THE RESPIRATION OF STREPTOCOCCUS PYOGENES AND PNEUMOCOCCUS TYPE I

III. Bearing of Respiration on Existing Theories of the Mechanism of the Action of the Chemotherapeutic Agents[1]

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Chemotherapeutic drugs to combat infectious disease have been sought since earliest times. It has not been until recently that real success has been attained, however. Most spectacular in degree of success has been that attained in the past five years, first with the dye prontosil and then with its derivatives of the sulfonamide group. The mechanisms of the action of these drugs have not as yet been understood. It would obviously be most desirable to establish use of the sulfonamide drugs on a basis of rational understanding of their mode of action.

Since the wide use of sulfanilamide and allied drugs, several theories have been proposed to account for their action. Long and Bliss (1939) have already discussed some of these theories and we therefore need not reconsider them here. We will review here the two theories which are related to the respiration.

I. ANTICATALASE THEORY

Despite the fact that no one has yet demonstrated that p-hydroxylaminobenzenesulfonamide results from the oxidation of sulfanilamide *in vitro*, or *in vivo*, various theories (Locke, Main and Mellon, 1938; Main, Shinn and Mellon, 1938; Shinn, Main and Mellon, 1938, 1939; Schaffer, 1939; Fox, German and Janeway, 1939; Fox, 1940) based on Mayer's[2] assumption have been put forward to explain the mechanism of sulfanilamide action.

[1] This work has been supported by a grant from The Commonwealth Fund.

[2] See the preceding article by M. G. Sevag and Myrtle Shelburne.

Of these theories the "anti-catalase" theory (Locke, Main, Shinn, Mellon, 1938, 1939) will be considered in the light of our experimental findings. Sevag and Maiweg (1934b, 1936) reported that under certain conditions oximes, and neutral solutions of hydroxylamine hydrochloride (Jacobson, 1892; Blaschko, 1935) exercise a powerful inhibiting effect on catalase. These findings have been used as a basis for several speculations (Locke, *et al.*). It has been stated that hydroxylaminobenzenesulfonamide, assumed to be formed from sulfanilamide, exercises an inhibiting effect on catalase similar to that of oximes and hydroxylamine hydrochloride. This assumed inhibiting effect on catalase in the animal system is supposed to cause the accumulation of hydrogen peroxide resulting from the metabolism of streptococci or pneumococci; the hydrogen peroxide in turn destroys the bacteria. The therapeutic property of sulfanilamide is attributed to this supposed chain of events according to the "anti-catalase" theory.

The chemical properties of the hydroxylaminobenzenesulfonamide on one hand, and the oximes and hydroxylamine hydrochloride on the other are entirely different. Furthermore, the assumed similarity with respect to anticatalase activity could not be demonstrated. Numerous experiments (table 1, systems 7, 8, 11, 12; table 3, systems 5, 6) pertinent to this question were carried out and in no case did the pure hydroxylaminobenzenesulfonamide exercise any inhibiting effect on the catalase.

The proponents of the "anti-catalase" theory based their assumptions on results obtained in an indirect manner. Irradiation of sulfanilamide was assumed to oxidize it to hydroxylaminobenzenesulfonamide which in turn inhibited catalase. Under the conditions of irradiation this substance, if ever formed, would be readily oxidized. The anticatalase activity of irradiated sulfanilamide solutions must, therefore, be due to a different cause.

Benoy (1940) showed that sulfanilamide acquired anticatalase activity after irradiation in an atmosphere of nitrogen, and that oxygen was not essential, which refutes the idea that sulfanilamide is oxidized to hydroxylaminobenzenesulfonamide by irradiation. She further stated that the irradiation of $C_6H_5SO_2NH_2$, $C_6H_5SO_3Na$, and C_6H_6 makes these compounds acquire a catalase-in-

hibiting property. Since, structurally, they are incapable of giving rise to hydroxylamino-derivatives she suggested that the benzene ring itself in some way is activated to exercise anticatalase activity.

Complete inhibition of the aerobic respiration of streptococci in both the presence and absence of hydrogen peroxide resulting from the oxidation of p-hydroxylaminobenzenesulfonamide. During the aerobic respiration of streptococci the accumulation of detectable amounts of hydrogen peroxide theoretically believed to be formed from the oxidation of the substrate, could not be demonstrated. Moreover the inhibition of respiration by sulfanilamide in the presence and absence of catalase was of the same degree. On the other hand during aerobic respiration in the presence of hydroxylaminobenzenesulfonamide the accumulation of hydrogen peroxide resulting from the oxidation of the drug could be easily shown and measured. When corrections were made for the blank, about 95 per cent inhibition of respiration was obtained. In parallel experiments in the presence of catalase where hydrogen peroxide was completely eliminated, the inhibition was likewise 95 per cent (table 1). These facts show that hydroxylaminobenzenesulfonamide is responsible for the inhibition of aerobic respiration in the presence and absence of catalase, or in the presence and absence of small amounts of hydrogen peroxide.

Inhibition of the respiration of pneumococci Type I: Inhibition of the aerobic respiration of pneumococci by sulfanilamide in the presence and absence of hydrogen peroxide resulting from the oxidation of glucose. In contrast to streptococci, the accumulation of measurable amounts of hydrogen peroxide during the aerobic respiration of pneumococci was easily demonstrable (Sevag, 1933a and b; Sevag and Maiweg, 1934a). A glance at table 2 shows that the respiration of pneumococci produces hydrogen peroxide. In the presence of catalase, hydrogen peroxide was absent. Whether in the absence or presence of catalase the inhibition by sulfanilamide is of the same magnitude.

The respiration in the absence of yeast extract and serum is weak; the inhibition by sulfanilamide under these unfavorable conditions is rather uncertain (table 2, systems 1 and 2). Evi-

TABLE 1

Inhibition of the aerobic respiration of Streptococcus pyogenes (strain C203M) by p-hydroxylaminobenzenesulfonamide in the presence and absence of catalase

Each system contained: 3 mgm. of yeast extract (0.5 ml.), normal horse serum (0.5 ml.).

The drug (10 mgm.) and the glucose (10 mgm.) were added in dry form by tipping in the side arm of the flask. The streptococci were suspended in phosphate buffer of pH 7.4 (0.4 ml.). The systems which did not contain organisms were given equivalent volume of phosphate. Catalase was added in 0.2 ml. amounts. The final volume of each system was made to 2.8 ml. with saline.

(a) Cmm. O_2 consumed/1.63 mgm. strep./150 minutes.

(b) Cmm. O_2 consumed/1.56 mgm. strep./150 minutes.

NUMBER	AEROBIC RESPIRATION SYSTEMS		CMM. O_2 CONSUMED (ACTUAL MEASUREMENTS)	CMM. O_2 CONSUMED BY STREPTOCOCCI (CORRECTED VALUES)	ML. OF 0.005 N $Na_2S_2O_3$ USED TO TITRATE H_2O_2	CMM. O_2 FOUND AS H_2O_2	PER CENT INHIBITION OF RESPIRATION BY DRUG
1	Streptococci		(a) 144 (b) 70	144 70	0 0	0 0	
2	Streptococci	Catalase	(a) 144 (b) 76	144 76	0 0	0 0	
3	Streptococci	Glucose	(a) 232 (b) 249	232 249	0 0	0 0	
4	Streptococci	Glucose Catalase	(a) 234 (b) 263	234 263	0 0	0 0	
5	Drug		(a) 533 (b) 542		7 8	392 448	
6	Streptococci Drug		(a) 535 (b) 537	2 0	5.8 6.5	325 364	99* 100
7	Drug	Catalase	(a) 321 (b) 366		0 0	0 0	
8	Streptococci Drug	Catalase	(a) 334 (b) 324	13 0	0 0	0 0	91† 100
9	Drug	Glucose	(a) 504 (b) 578		6.6 7.8	370 437	
10	Streptococci Drug	Glucose	(a) 555 (b) 574	51 56	4.8 6.2	269 347	78‡ 77
11	Drug	Glucose Catalase	(a) 277 (b) 278		0 0	0 0	
12	Streptococci Drug	Glucose Catalase	(a) 330 (b) 309	53 31	0 0	0 0	78§ 88

* Compared with system No. 1.

† Compared with system No. 2.

‡ Compared with system No. 3.

§ Compared with system No. 4.

TABLE 2

Inhibition of aerobic respiration of pneumococcus (type I) by p-aminobenzenesulfonamide in the presence and absence of catalase

Cmm. O_2/mgm. pneumococci*/150 minutes

NUMBER	AEROBIC RESPIRATION SYSTEMS	CONTROL			SULFANILAMIDE 0.04M			PER CENT INHIBITION
		Cmm. O_2 consumed by pneumococci	Ml. of 0.005N $Na_2S_2O_3$ used to titrate H_2O_2	Cmm. O_2 found as H_2O_2	Cmm. O_2 consumed by pneumococci	Ml. of 0.005N $Na_2S_2O_3$ used to titrate H_2O_2	Cmm. O_2 found as H_2O_2	
	ml.							
1	Pneumococci.....0.4	(a) 48	0.50	28	48	0.50	28	0
	Phosphate buffer pH 7.4.....5.0	(b) 55	0.36	20	43	0.29	16	22
	Glucose 0.1 M.....0.4							
2	Pneumococci.....0.4	(a) 90†	0	0	84†	0	0	7
	Phosphate buffer pH 7.4.....4.8	(b) 102	0	0	98	0	0	0
	Glucose 0.1 M.....0.4							
	Catalase.....0.2							
3	Pneumococci.....0.4	(a) 291	2.1	118	219	1.7	95	25
	Phosphate buffer pH 7.4.....4.0	(b) 330	3.1	174	207	1.8	101	37
	Glucose 0.1 M.....0.4							
	Serum (catalase free).....0.5							
	Yeast extract, 3 mgm.....0.5							
4	Pneumococci.....0.4	(a) 440†	0	0	326†	0	0	26
	Phosphate buffer pH 7.4.....4.0	(b) 512	0	0	332	0	0	35
	Glucose 0.1 M.....0.4	(c) 1,210‡	0	0	692	0	0	42
	Serum (catalase free).....0.5							
	Yeast extract, 3 mgm.....0.5							
	Catalase.....0.2							

* Based on the total nitrogen value 12.2 per cent analyzed by a method described for streptococci (see article I).

† These values calculated from the manometric readings were multiplied by 2 because of the reaction between catalase and H_2O_2. $2H_2O_2$ + catalase → $2H_2O$ + O_2. The O_2 thus liberated returns to the system and creates one volume of positive pressure and reduces the negative pressure proportionately.

‡ This higher figure may be accounted for by the fact that the organisms used in this experiment (c) were washed in phosphate buffer containing catalase and were suspended in the same. The organisms of experiments (a) and (b) were washed in phosphate buffer alone.

dently under these conditions the drug is only mildly effective. In the presence of yeast extract and serum the respiration is greatly accelerated and the inhibition is 29 per cent. In one experiment (system 4) the oxygen uptake in the presence of catalase was 1210 cmm. and the inhibition 42 per cent. From these and other data it would follow that the greater the respiration, the greater the inhibition by sulfanilamide.

The above data once more show that the respiration of pneumococci is inhibited by sulfanilamide irrespective of the presence or absence of catalase, and likewise irrespective of the absence of, or the accumulation of, small amounts of hydrogen peroxide during respiration.

Complete inhibition of the aerobic respiration of pneumococci by p-hydroxylaminobenzenesulfonamide in the presence and absence of hydrogen peroxide resulting from the oxidation of glucose and the drug. In the absence of catalase the amount of hydrogen peroxide accumulated was nearly twice as much when p-hydroxylaminobenzenesulfonamide was present during respiration instead of sulfanilamide (compare tables 2 and 3). When we added 0.2 ml. of water-clear catalase solution (= 0.02 ml. whole blood) to the systems not a trace of hydrogen peroxide could be detected after a respiration period of 150 minutes. In the absence of catalase the inhibition by the drug was 89 to 95 per cent, and in the presence of catalase it was 85 to 86 per cent (table 3). Evidently the amount of hydrogen peroxide accumulated in the catalase-free system did not accentuate the inhibition by the drug. It was also clearly seen that under conditions favorable for active respiration the accumulated hydrogen peroxide did not appear to exercise a marked toxic effect. In system 1 (table 3) we see that respiration continued until an amount of hydrogen peroxide corresponding to 1.85 to 4.5 ml. of 0.005 N $Na_2S_2O_3$ was accumulated; this corresponds to a concentration of the accumulated hydrogen peroxide of 0.0008 to 0.0017 M.

After 105 minutes of respiration, experiments were continued for another period of 60 minutes. During the latter period the system continued to use oxygen at the rate of 94 cmm. oxygen per hour per milligram pneumococci despite the fact that the system contained 0.0008 to 0.0017 M hydrogen peroxide. The toxic

effect of hydrogen peroxide appears to be rather limited in an environment still rich in nutrient.

TABLE 3

Inhibition of the aerobic respiration of pneumococcus (type I) by p-hydroxylaminobenzenesulfonamide in the presence and absence of catalase

Each system contained 3 mgm. of yeast extract (0.5 ml.), normal horse serum (catalase free) (0.5 ml.), and 0.3 M glucose (0.4 ml.).

The drug (10 mgm.) was added in dry form by tipping in the side arm of the flask. The pneumococci were suspended in phosphate buffer of pH 7.4 (0.4 ml.). Catalase was added in 0.2 ml. amounts. The final volume of each system was made to 5.8 ml. with buffer.

(a) Cmm. O_2 consumed/0.89 mgm. pneu./165 minutes.

(b) Cmm. O_2 consumed/1.45 mgm. pneu./150 minutes.

NUMBER	AEROBIC RESPIRATION SYSTEM	CMM. O_2 CONSUMED (ACTUAL MEASUREMENTS)	CMM. O_2 CONSUMED BY STREPTOCOCCI (CORRECTED VALUES)	ML. OF 0.005N $Na_2S_2O_3$ USED TO TITRATE H_2O_2	CMM. O_2 FOUND AS H_2O_2	PER CENT INHIBITION OF RESPIRATION BY DRUG
1	Pneumococci	(a) 256 (b) 478	256 478	1.85 4.50	104 252	
2	Pneumococci Catalase	(a) 194 (b) 372	388‡ 744	0 0	0 0	
3	Drug§	(a) 244 (b) 280		2.0 3.3	112 185	
4	Pneumococci Drug	(a) 280 (b) 305	36 25	2.3 3.3	129 185	89* 95
5	Drug Catalase	(a) 165 (b) 179		0 0	0 0	
6	Pneumococci Drug Catalase	(a) 193 (b) 235	56‡ 112	0 0	0 0	86† 85

* Compared with system No. 1.

† Compared with system No. 2.

‡ These values were multiplied by 2 in order to correct for the hydrogen peroxide decomposed by catalase. See table 2, footnote †.

§ Melting point 161°C.

The data also show that 10 mgm. of p-hydroxylaminobenzenesulfonamide is incapable of inhibiting 0.2 ml. of water-clear catalase solution. This corresponds to 0.02 ml. of defibrinated whole

blood. In other words, if 250 grams of p-hydroxylaminobenzenesulfonamide could be injected into a 10 kgm. dog with 500 ml. of blood (5.5 per cent of the body weight) without causing death, it should not inhibit the blood catalase to a measurable degree. This amount of drug is at least 100 times the fatal dose.

II. FILDES THEORY ON THE ACTION OF ANTIBACTERIAL DRUGS

Fildes (1940) formulated his theory as follows: "Antibacterial substances function by 'interfering' with an essential metabolite and thus inhibit growth." Sulfanilamide inhibits by competing for an enzyme associated with the essential metabolite. "An 'essential metabolite' is a substance or chemical group which takes an essential part in a chain of syntheses necessary for bacterial growth. A 'growth factor' which must be supplied in the nutrients is an essential metabolite which the cell cannot synthesise. Nicotinic acid, for instance, is an essential metabolite for all bacteria but a growth factor for only a few."

Woods (1940) found that p-aminobenzoic acid in high dilutions reversed the action of sulfanilamide in a manner similar to a fraction obtained from yeast extract. Fildes then supposed that p-aminobenzoic acid is an essential metabolite normally associated with an enzyme. "Sulfanilamide being structurally similar to it, is capable, if in sufficient concentration, of displacing p-aminobenzoic acid from its enzyme and stopping this essential line of metabolism."

"On this view the 'sensitivity' of a microbe to sulfanilamide would depend at least in part upon whether it could synthesise p-aminobenzoic acid readily or not. An organism whose synthetic powers were poor would be more sensitive than one with greater power. Similarly a large number of bacteria would be less affected by a certain concentration of sulfanilamide than a small number. Inhibition or not would become a question of the proportion of sulfanilamide to p-aminobenzoic acid affecting the enzymes of each cell."

Our experimental findings are at variance with the above view expressed by Fildes regarding the role of p-aminobenzoic acid. We have found that 0.012 to 0.035 molar p-aminobenzoic acid, in the absence of sulfanilamide, inhibits the aerobic and anaerobic

respiration of streptococci to the extent of 10 to 50 per cent during a 1 to 3 hour period. In growth experiments, observed after 2-, 8-, 19-, and 23-hour periods, 0.035 M p-aminobenzoic acid *per se* inhibited the growth 22, 34, 63, 67 per cent, respectively, and with 0.006 M concentration this effect was, respectively, 0, 0, 23, and 20 per cent. Lower concentrations neither inhibited nor accelerated growth. The inter-action of sulfanilamide and p-aminobenzoic acid on the growth of streptococci is being further studied. We can, however, state that 0.012 to 0.035 M p-aminobenzoic acid exercises a continuous additive inhibiting effect on the respiration of streptococci in the presence of sulfanilamide. The inhibiting effect of sulfanilamide on respiration may sometimes be nearly completely or partially reversed by 0.006 to 0.0006 molar p-aminobenzoic acid, but at other times it has no effect whatsoever. In the absence of sulfanilamide, there is observed occasional acceleration of respiration in the early stages (15 to 30 minutes) with 0.006 to 0.0006 M p-aminobenzoic acid. This, however, slows down and very rarely exceeds the normal respiration after a period of 1 to 3 hours.

Fildes stated that p-aminobenzoic acid had neither been isolated from bacteria nor yeast, nor had it been proved to be a growth factor. On the other hand, Rubbo and Gillespie (1940) believe that they have isolated the benzoyl derivative of p-aminobenzoic acid from yeast. They obtained 750 mgm. of yeast concentrate from 30 kgm. of wet brewer's yeast. An ether extract of this concentrate was benzoylated and the product recrystallized five times, melted at 277°C. They considered this product as p-benzoyl-aminobenzoic acid. The yield was 2 mgm. No mixed melting point determination, or other tests were reported. As numerous different substances may have the same melting point the question as to whether p-aminobenzoic acid is normally present in living cells must, for the present, be left open.[3] They also reported that p-aminobenzoic acid is a growth factor for *Clostridium acetobutylicum* in a restricted medium containing asparagine, glucose and salts only.

The data presented by Rubbo and Gillespie are, however, dis-

[3] After submitting these articles to the press Blanchard (1941) reported the isolation and characterization of p-aminobenzoic acid from yeast.

concerting and throws doubt on the supposition that p-aminobenzoic acid is a growth factor. The reported data show that when p-aminobenzoic acid was present in amounts of from 2×10^{-5} to 2×10^{-1} micrograms there was growth, but in quantities of 2 micrograms there was no growth. Novocaine in amounts of from 2×10^{-4} to 2×10^{-1} micrograms supported growth, but growth was absent when novacaine was present in either 2×10^{-5} or 2 microgram amounts. Therefore, growth could be inhibited or supported merely by an increase or decrease of 2 micrograms or less of p-aminobenzoic acid or novocaine.

p-Aminobenzoic acid in amounts from 1×10^{-3} to 5×10^{-3} micrograms failed to neutralize the inhibiting effect of M/1650 sulfanilamide. p-aminobenzoic acid in two amounts, 1×10^{-3} and 5×10^{-3} micrograms, likewise failed to neutralize the inhibiting effect of M/3300 sulfanilamide, but the inhibiting effect of M/3300 sulfanilamide was neutralized by 2×10^{-3}, 3×10^{-3}, and 4×10^{-3} micrograms of p-aminobenzoic acid. From the above it is seen that a difference of 1×10^{-3} micrograms of p-aminobenzoic acid was sufficient to cause either inhibition or neutralization of the inhibition of growth by sulfanilamide. The restriction of the supposed growth function, and also of the neutralization action on the inhibiting effect of sulfanilamide by p-aminobenzoic acid to a "zone of limited concentration" is unusual for substances known to be bacterial growth factors or "essential metabolites." This is worthy of notice and difficult to explain by assuming that p-aminobenzoic acid functioned as a growth factor.[4]

The fact that an increase of 2 micrograms of p-aminobenzoic acid caused it to be ineffective as a growth factor suggests that, in these instances, it must have acted as an inhibitor of growth. This is in agreement with our findings. We also found that, as stated above, the smaller quantities neutralized the inhibiting effect of sulfanilamide on respiration and that slightly larger quantities inhibited markedly in the absence of, and increased additively inhibition in the presence of, sulfanilamide. A quan-

[4] After submitting their articles to the press Lampen and Peterson (1941) reported that they were unable to confirm the conclusion of Rubbo and Gillespie.

tity of p-aminobenzoic acid which neutralized the inhibiting effect of sulfanilamide did not increase the respiration and growth over that of the normal.

In the light of these facts it would seem that the action of p-aminobenzoic acid has a physico-chemical basis rather than a physiological one. The growth reported in the presence of a "restricted zone of concentration" of p-aminobenzoic acid may therefore be interpreted by assuming that these quantities do not support growth but are sufficient to displace (and not enough to inhibit), from the bacterial cell specific reversible inhibitors usually formed during growth, thus enabling the organisms to grow. The formation of inhibitors resulting from the enzymic reactions is widely known.

The question whether p-aminobenzoic acid is a growth factor or not may also be viewed from another angle. It is well known that bacterial enzymes are of adaptive nature; variations in morphology, cellular substance and enzyme activities are thus brought about. Under restricted nutritional conditions the cells may utilize substances which they ordinarily would not use. The processes of growth and their inhibition by drugs under these conditions could not be compared with the processes of growth under favorable conditions. Taking these facts into consideration, we found in all our experiments that sulfanilamide inhibits respiration in the absence of growth, and also respiration, and thereby growth, in systems containing optimal amounts of yeast extract and serum.

As the materials used in the above respiration and growth systems have been utilized by Woods and others to obtain fractions comparable in effect to that exercised by p-aminobenzoic acid, its supposed rôle as a growth factor or an "essential metabolite" to cause the neutralization of sulfanilamide action would seem to us difficult to support. Furthermore, the fact that it can exercise this assumed rôle only with quantities within a highly "restricted zone of concentration" above which an increase of a few micrograms causes it to act as an inhibitor, suggests that the mechanism of its action must lie in some other action, as discussed below.

III. INHIBITION OF RESPIRATION THEORY

Our findings show clearly that sulfanilamide exercises inhibition on respiration not involving any growth activity. The oxidation products of glucose resulting from the respiration of streptoccoci may or may not be utilized for synthetic processes necessary for growth, but the energy thus liberated is necessary for growth processes. Sulfanilamide blocks primarily the processes which provide energy before, and independent of, the start of the synthetic processes necessary for growth.

In the presence of glucose, and absence of yeast extract or serum or both, the anaerobic respiration of washed streptococci was inhibited from 20 to 92 per cent (article II, table 2, system 1).[5] Anaerobic and aerobic respiration in the presence of yeast extract alone were likewise inhibited from 12 to 30, and 40 to 49 per cent, respectively (article II, tables 1 and 2, system 2). Under these conditions no growth takes place (article II, table 3). Under conditions favorable for growth the inhibition of respiration results in the immediate and proportional inhibition of growth (article II, table 4).

In living cells respiration is the manifestation of reversible oxidation-reduction reactions, whereby substrate hydrogen atoms "activated" by dehydrogenases are transferred. The transfer seems to occur on a surface (the protein bearer of dehydrogenases) on which both the acceptor and the donator are adsorbed and thereby the reactions are catalyzed. The so-called co-enzymes are the actual prosthetic groups which combine with the proteins to form the catalytically active complexes. The most important property of these co-enzymes is that they may combine with different protein bearers forming reversible oxidation-reduction enzyme systems of different specificity. Another important property of the co-enzyme-protein complexes is that they are dissociable, and in certain cases their conjugation with protein depends on the state of oxidation-reduction (Negelein and Wulff, 1937). Co-enzyme I and co-enzyme II are phosphopyridine

[5] References in this paragraph are to preceding article by Sevag and Shelburne (J. Bact., **43**, 411–421).

nucleotides, differing in the number of the phosphoric acid radicals. Alloxazine-adenine dinucleotide is the coenzyme of Haas' Flavoprotein (Haas, 1938). Co-carboxylase is the prosthetic group of carboxylase, also a coenzyme-protein complex.

In view of the fact that the enzymes possess protein structure, they are affected by the different protein reagents; e.g., heavy metals, alkaloid reagents, heat, etc. Such inhibition is often irreversible and is considered non-specific. On the other hand there exist a large number of substances which appear to inhibit selectively a given enzyme reaction. Such inhibition is considered to be specific, and is often reversible. The chemical and physical differences between the specific proteins of the different enzymes, the different coenzymes, etc., make specific inhibition possible (Elvehjem and Wilson, 1939; Oppenheim and Stern, 1939; Tam and Wilson, 1941). Since most of the specific inhibitors have a chemical basis for their effects, it should be possible to comprehend the mechanism of inhibition related to the action of chemotherapeutic agents.

It would perhaps seem logical therefore to assume that the chemotherapeutic substances which have structural similarity to the whole, or part of the coenzyme molecules may combine specifically with the protein bearer of the respiration enzymes. This combination may take place as a result of the displacement of coenzymes by the drug or by a reversible union of the drug as a second prosthetic group with the protein in a state which inhibits in some manner the oxidation and reduction of the coenzymes I and II, and that of Haas.

Sulfapyridine with its pyridine ring may be looked upon as a potential competitor of coenzymes I, II, and sulfathiazole of co-carboxylase. An analogy might be drawn between this view and the results obtained by Dorfman, Rice, Koser, and Saunders (1940). They showed that when nicotinamide was added first to the respiration system of dysentery bacilli, the inhibition by sulfapyridine was 13.3 per cent. When sulfapyridine was added first, 86.6 per cent inhibition was obtained which was not neutralized appreciably on the addition of nicotinamide. Though the action of sulfathiazole on co-carboxylase activity has not as

yet been studied, Buchman, Heegaard, and Bonner (1940) made the interesting observation that thiazole pyrophosphate markedly inhibited the activity of co-carboxylase. Neither thiazole, nor pyrophosphate exercised any inhibiting effect. The union between thiazole pyrophosphate and the carboxylase protein is stated to be through the pyrophosphate group which is common to both the co-carboxylase and the inhibitor. These facts would seem to indicate with a great deal of certainty that the inhibitors are bringing about the formation of either an inactive "enzyme analogue" or "drug-protein-coenzyme complexes." These being incapable of oxidizing glucose, bacterial respiration and growth are inhibited.

In presenting the above concept we realize, however, its insufficiency to account for the inhibition of respiration by sulfanilamide which is not structurally similar to the known coenzymes. Since this concept cannot likewise explain the action of some other anti-infectious chemotherapeutic agents, this cannot be the complete answer. As each bacterium contains specific enzyme proteins, the nature of their affinity for a substance may be the determining factor in the type of substance that will inhibit and act as a chemotherapeutic agent. A reasonable assumption to explain the action of sulfanilamide would be a mutual affinity between the drug and the proteins of the enzyme complex. Such an affinity does not necessarily arise from a similarity between the structure of a drug and the coenzymes, but may be the result of an affinity analogous to that existing between a coenzyme and its specific protein, the consequence being the displacement of the coenzymes by the drug and the formation of an inactive "enzyme analogue" or in the formation of a hypothetical "drug-protein enzyme" complex. This would be somewhat analogous to the "poisoning" of specific metal surface catalysts by substances having affinities for the catalytic surfaces which are stronger than that of the reagents which the catalysts activate in the particular reaction (Taylor, 1925).

The inhibition of the respiration and growth of streptococci by sulfanilamide, as well as the inhibition of respiration by higher concentrations of p-aminobenzoic acid may be explained by the

above concept. The reversal of the sulfanilamide action by very high dilutions of p-aminobenzoic acid can also be explained in the same manner. In the latter case, the displacement of sulfanilamide from the enzyme surface brought about by high dilutions of p-aminobenzoic acid does not appear to result in the continuation of the inhibition caused by sulfanilamide.

SUMMARY

Experiments have been carried out in relation to the existing theories of the action of sulfanilamide and related substances.

In the light of our findings the "anti-catalase" theory is untenable. We feel that our findings offer support to the theory of Fildes to the extent that sulfanilamide interferes with or blocks a bacterial enzyme system. We believe, however, that sulfanilamide blocks primarily the respiratory enzymes, which in turn inhibits growth, rather than interfering with the synthetic processes necessary for growth as stated by Fildes.

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