state, it does not explain the antibacterial and antifungal potencies of 2-methyl-3-methoxy-1,4-naphthoquinone, because this quinone has no free position on the quinone side of the molecule. Oxford (*9*) has shown that among p-benzoquinone derivatives the spinulosin-trimethylether (trimethoxytoluquinone) is one of the strongest bacteriostatics of this group; certainly, no possibility of addition exists in this compound. The effect of excess amounts of sulfhydryl compounds on the bacteriostatic potency of some quinones does not prove conclusively the existence of a specific antagonism between quinones and —SH groups. The inactivation of the quinones can be achieved by excess amounts of other strongly reducing substances (e.g. sodium bisulfite) as well.

Quinones are very reactive substances. In experimental work done in this laboratory (*6*), the inhibitory potency of many quinone derivatives on some enzyme systems (urease, papain, catalase, etc.) was tested. It was found that some of the very strongest antibiotics had no, or nearly no, effect on the activity of these enzymes. In the case of urease and papain, certainly —SH groups, which function as activating groups for the enzymatic process, are involved. A certain parallelism between antibiotic properties and inhibitory potency on urease and papain should be expected, if really the reaction with sulfhydryl compounds would be the dominant mechanism of the antibiotic action. By the same experiments it was shown that some enzymes, in which certainly no sulfhydryl groups play any role, also are inhibited by quinones. Of course, the inhibition of enzymes without —SH groups also could be one of the means by which the quinones exert their antibiotic effects. It is also known that quinones can react with amino groups of proteins and amino acids; the tanning properties of p-benzoquinone are used in industry to a certain extent (*5*). It could be imagined that, by tanning the protein compounds of the bacterial cellular membranes, normal cell division could be inhibited. Another possible mechanism can be derived from the relatively elevated oxidation-reduction potential of most of the quinones. Wieland (*10*) has demonstrated that p-benzoquinone is a strong inhibitor of some dehydrogenases. Thus, quinones could act as antibiotics by interfering with bacterial respiratory enzymes and thereby inhibit the synthesis of essential cell components.

The theory that the mode of antibiotic action of the quinones is a complex one can be supported by the fact that some of the quinones with strongest bacteriostatic potency on *Staphylococcus* have very little effect on *Escherichia coli* or on some species of yeast as *Saccharomyces cerivisiae* or *Torula utilis* (*7*), while other quinone derivatives, whose toxicity on *Staphylococcus* is quite negligible, exert rather strong effects on the other organisms mentioned. In experiments with *Planaria gonocephala* we were able to show that, using one compound, it was possible to distinguish two different dominant factors, progressing from higher to lower concentrations of the toxic agent. The data obtained lead us to believe that the effect of higher concentrations of the quinones is dominantly due to the tanning property of these compounds, while in lower concentration the quinones exert their antibiotic effects by the inhibition of some enzyme of the organism, most probably some of the oxidative-reductive enzymes.

The experiments mentioned suggest clearly that the antibiotic action of the quinones is not due to a single reaction, but to a very complex mechanism. The mode of action proposed by Colwell and McCall is certainly only one of the means by which the quinones exert their effects and, in most cases, seems not to be the dominant one.

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Virus Hemagglutination[1]

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The phenomenon of virus specificity is a familiar biological problem, but, with the possible exception of the bacteriophage group of viruses, the nature of the factors responsible for this specificity has not been clearly defined. With respect to the bacteriophage there is evidence that the virus unites with a specific component on the cell—in the case of *coli* and coliphage, for instance, with an antigenic surface polysaccharide (*5*). In the animal virus group, Curnen and Horsfall have shown that the P.V.M. virus can unite with some cellular component of lung tissue (*2*). The combining material was described as tissue particles.

It is evident that accurate characterization of the specific combining tissue components, especially with respect to their structural chemistry, might lead to an understanding of virus specificity in terms of chemical or metabolic relations between the virus and cell. In this connection it was thought that one possible approach to the problem would be the elucidation of the factors responsible for the hemagglutination phenomenon first described by Hirst (*3*) and studied further by Burnet, McCrea, and Stone (*1*) with respect to the alteration of cell receptors by virus action. The results of some preliminary studies along these lines are the subject of this communication.

From red blood cells[3] which are specifically agglutinated by certain viruses it has been found possible to prepare lipid

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[3] Human red cell residues from plasma fractionation were kindly supplied by the Antitoxin and Vaccine Laboratory, Massachusetts Department of Public Health.

extracts capable of inhibiting the hemagglutinating action of the same viruses for these cells. The effect is presumably due to competitive combination of the extract with the virus. Comparison of Rows 4, 5, 7, and 8 with 1 and 2, and Rows 13 and 14). On the other hand, both human and sheep inhibitors interfered with the action of mumps virus[4] on human and chicken cells (compare Rows 7 and 4 with 1 and Rows 8 and 5 with 2). Both human and sheep inhibitors also inter-

TABLE 1

Row	Virus	Inhibitor source	Red cells	Dilution of virus								
				1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
1	Mumps	Saline	Human	+++	+++	+++	+++	+++	++	−	−	−
2	"	"	Chicken	+++	+++	+++	+++	+++	+++	+	−	−
3	"	"	Sheep	+++	+++	+++	+++	+++	+++	+	−	−
4	"	Sheep R.B.C.	Human	+	+	±	−	−	−	−	−	−
5	"	"	Chicken	+++	+++	+++	+++	+	−	−	−	−
6	"	"	Sheep	+++	+++	+++	+++	+++	+++	+	−	−
7	"	Human R.B.C.	Human	−	−	−	−	−	−	−	−	−
8	"	"	Chicken	−	−	−	−	−	−	−	−	−
9	"	"	Sheep	+++	+++	+++	+++	+++	+++	−	−	−
				1/25	1/50	1/100	1/200					
10	"	Sheep #2	Sheep #2	+	−	−	−					
11	"	Human	Sheep #2	+	−	−	−					
12	"	Saline	Sheep #2	+++	+++	+	−					
				1/1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
13	PR 8	Saline	Human	+++	+++	+++	+++	+++	+++	+++	+++	+
14	"	Saline	Chicken	+++	+++	+++	+++	+++	+++	+++	+++	+
15	"	Sheep R.B.C.	Human	+++	+++	+++	+++	+++	+++	+++	+++	−
16	"	"	Chicken	+++	+++	+++	+++	+++	+++	++	++	+
17	"	Human R.B.C.	Human	++	±	−	−	−	−	−	−	−
18	"	"	Chicken	−	−	−	−	−	−	−	−	−

17 and 18 with 13 and 14 in Table 1, indicates the extent of inhibition in a typical experiment. In these experiments 0.2 cc. of saline emulsion of the inhibiting material was mixed with serial dilutions (0.2 cc.) of allantoic fluid of chick embryos infected with virus, and the mixture was allowed to stand at 2° C. for 30 minutes. One-tenth cc. of 2.5 per cent suspension of red cells was then added, the mixture was thoroughly shaken, and the cells were allowed to settle. The degree of agglutination was read by the method of Salk (*6*).

The inhibition reactions thus far tested seem to demonstrate specificity corresponding to that shown by the viruses in their hemagglutinating actions. Thus, it is known that influenza virus will agglutinate human and chicken red blood cells, but not sheep cells, whereas mumps virus will agglutinate human, chicken, and sheep red cells. Assuming structural similarity or identity between the cell receptor and the inhibitor molecule, it would then be expected that human inhibiting extract would be active against both influenza and mumps viruses, but that sheep inhibitor would only be active against mumps virus and inactive against influenza virus. Corresponding to these expectations it was found that human inhibitor prevented the hemagglutinative action of influenza on both human and chicken red cells (compare Rows 17 and 18 with 13 and 14 in Table 1), whereas sheep inhibitor had no effect on the influenza virus (compare Rows 15 and 16 with

fered with the action of mumps virus on sheep cells, but this effect was often weaker and not obtained as invariably as were the other inhibitory effects (compare Rows 9 and 6 with 3, and Rows 10 and 11 with 12).

TABLE 2

Time of contact before removal of aliquot	Final dilution of virus							
	1/2.5	1/5	1/10	1/20	1/40	1/80	1/160	1/320
1 min.	−	−	−	−	−	−	−	−
30 "	+	−	−	−	−	−	−	−
60 "	++	−	−	−	−	−	−	−
120 "	++	+	−	−	−	−	−	−
240 "	+++	++	+	±	−	−	−	−
Virus at room temperature 4 hrs.	+++	+++	+++	+++	+++	+++	++	−
Inhibitor at room temperature 4 hrs.	−	−						

+ = agglutination.

On prolonged contact of virus and inhibitor at 25° C. or 37° C., interaction between the two resulted in progressive inactivation of the inhibitor, evidenced by reappearance of increasing hemagglutinative activity in an inactive mixture (Table 2). In this experiment influenza virus and human in-

[4] Egg adapted strain kindly supplied by J. F. Enders.

hibitor were mixed and allowed to stand at room temperatures. Aliquots were removed at various intervals, serially diluted, and tested for hemagglutinating activity on human cells. The results are reminiscent of Hirst's description of reversal of hemagglutination (*4*) of cells, accompanied by elution of active virus and loss of cell susceptibility to further agglutination by the same virus. This similarity lends further support to the notion that the inhibitor is a derivative of, or identical with, the cell receptor. Influenza inhibitor has also been obtained from human lung.

Further details of this work and other related aspects of the problem, now under investigation, will be the subject of later communications.

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Reconsideration of the Photosynthetic Mechanism in *Chlorella*[1]

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Photosynthesis has been classically identified with the formation of carbohydrate. Theories of photosynthesis have frequently postulated a series of photochemical steps operating at all levels of reduction from carbon dioxide to carbohydrate. An a priori assumption in all such theories is that some form of carbohydrate is the direct product of the photochemical reactions. This viewpoint is consistent with data on photosynthesis in higher plants. For example, the elemental analysis of a corn plant indicates that its dry weight corresponds to almost pure carbohydrate (*3*); in short-time experiments on sunflower leaves the carbon dioxide taken up can be recovered quantitatively as carbohydrate (*6*).

Because of experimental advantages the green alga, *Chlorella pyrenoidosa*, has found wide use in studies on photosynthesis. No fundamental differences have been found between its photosynthetic behavior and that of higher plants. However, elemental analysis of the dry material of *Chlorella* is quite different than that of a higher plant, *i.e.* the nitrogen content is 8–10 per cent. As in the closely related colorless alga, *Prototheca* (*1*), the cell constituents may be considered to be about half protein and half carbohydrate. In close approximation, all the products of photosynthesis result in cellular materials. It follows that in a growing culture of *Chlorella* protein and carbohydrate syntheses must proceed at about equal rates.

The rapid synthesis of proteins (or other nitrogenous compounds) is borne out by studies of the assimilatory quotient as affected by the source of nitrogen supplied (nitrate or ammonium ion). Data obtained in manometric experiments by the usual procedures are summarized in Table 1.

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The assimilatory quotient is affected by the light intensity during measurement as well as by the nitrogen source. The effect is correlated with the following observations. Up to ~ 50 f.-c., rate of growth is proportional to light intensity and to the measured rate of gas exchange. At higher light intensities (e.g. ~ 600 f.-c.) the cells may show a much higher rate of gas exchange, but they do not grow any more rapidly than at 100 f.-c. (*4*). Although complete explanation of these

TABLE 1

Assimilatory Quotient (CO_2/O_2, Exchange Ratio) Under Light-limiting and Light-saturating Illumination as Affected by the Nitrogen Source

Nitrogen source	Illumination	
	40 f.-c.	600 f.-c.
NO_3^-	−0.5	−0.8
NH_4^+	−0.9	−0.9

phenomena is not yet available, it appears to be related to differences in the comparative rates of carbohydrate and protein syntheses which depend upon light intensity and the previous history of the cells. Discrepancies between the quotients reported here and those found by other workers may be explainable in the same way.

It has already been shown that starved cells of *Chlorella* will bring about an oxidative assimilation of acetic acid or glucose to what appears to be storage carbohydrate (*5*). This means that the processes of dark metabolism are able to effect synthesis of storage materials from respiratory intermediates. The accumulating products of photosynthesis also have been shown to be storage materials (*5*). Growing cells of *Chlorella* require protein and carbohydrate syntheses at approximately equal rates. These syntheses may result either from photosynthesis or from oxidative assimilation of an organic substrate in the dark. It is certainly reasonable to expect similarities in the pathways of the two modes of synthesis. We therefore offer, and shall further investigate, the following hypothesis: In photosynthesis the photochemical product is some intermediate, not itself carbohydrate, which may be converted subsequently to carbohydrate or to protein by metabolic pathways similar to those of heterotrophic forms.

We are well aware that this hypothesis has certain relations to, and apparent conflicts with, other theories and experimental data (e.g. *2*). There are also important implications of the low quotient at low light intensities with regard to past measurements of photosynthesis, in which a quotient of −0.9 has often been assumed. Experimental details, more complete data, and a consideration of their implications, will be reported elsewhere.

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