to the fact that vitamin C can be produced in the seeds by germination.

5. Because of the variation in vitamin C concentration in different tissues and the action of oxidase during dissection, the sampling error is considerably higher than the normal titration error, and if this is not taken into account it may seriously affect the results.

6. Very small tomatoes may, because of the higher proportion of skin, contain higher concentrations of vitamin C, but the differences found in tomatoes grown under the same conditions were not large enough to discredit the findings of Sansome &

Zilva on polyploidy, as McHenry & Graham had suggested.

7. Exposure to sunshine during ripening may affect the vitamin C content of tomatoes, but the latter is also affected by the oxidase, which may destroy a considerable amount of the vitamin if ripening takes place under conditions favouring oxidase action.

We are indebted to Sir Harry Hague and Mr H. H. Jones for samples of tomatoes grown under known conditions, to Dr C. D. Darlington for kindly examining seeds of our tomatoes for polyploidy, and to Miss M. Payne for assistance in preparing the extracts.

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The Anti-streptococcal Action of Iodinin. Naphthaquinones and Anthraquinones as its Main Natural Antagonists

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Chromobacterium iodinum was isolated and so named owing to the purple, bronze-glinting pigment which covers its colonies on suitable solid media [Davis, 1939]. This pigment, here termed 'iodinin', was found to inhibit the growth of certain other bacteria [McIlwain, 1941a]; and such may be its natural function. The organisms so inhibited include pathogens, and the mechanism of the inhibition has now been studied, since the compound is highly active and an understanding of such processes is of potential chemotherapeutic value.

Iodinin (I) is the di-*N*-oxide of a dihydroxy-phenazine. The positions of the hydroxyl groups

are unknown but Clemo & McIlwain [1938] have suggested formulae in which co-ordination or tautomerism can occur, and have excluded the 2:3- and 2:5-compounds. The *N*-oxidic structure has been confirmed by resynthesis of iodinin from the dihydroxyphenazine produced by its degradation [McIlwain, 1943].

Natural antagonists of the anti-streptococcal action of iodinin

Characterization of materials or conditions which alter the action of antibacterial agents have been of value in indicating the extent and limitations of the possible practical use of these agents. In addition, such studies have suggested the modes of action of some synthetic bacterial inhibitors and this understanding has led to the preparation of

Marmite, such inhibition was produced by $1.5-2 \times 10^{-6} M$ iodinin (Table 1). The relatively small difference between these minimal antibacterial levels permitted the use of the more complex media in subsequent work; this had the advantages of convenience and of making it more probable that the effects of reversing agents would be specific to the inhibitor (see p. 269).

In contrast to the inhibition caused by acriflavine or by the sulphonamides, inhibition by this pigment was relatively little affected by a variety of natural products, including microbial extracts, and plant and meat preparations (Table 2). The testing conditions employed little excess iodinin. This therapeutically valuable property may be due to limited distribution of materials related to the inhibitor, or to the type of inhibition which it produces [McIlwain, 1940b]. Blood (plasma or corpuscles),

Table 1. *Effect of medium on inhibition of streptococcal growth by iodinin*

Medium		Iodinin added $M \times 10^{-7}$	Growth after				
Type (see p. 269)	Addenda (mg./ml.)		18-24 hr.	28-30 hr.	2 days	4 days	7 days
Amino-acid (a)		0	++	++++	—	—	—
"		3	+	+++	++++	—	—
"		6	0	+	++++	—	—
"		12 and more	0	0	0	0	0
Peptone (b) or marmite (c)		0	++++	—	—	—	—
"		3	+	++++	—	—	—
"		6	+	++	++++	—	—
"		12	0	0	0	++++	—
"		25 and more	0	0	0	0	0
(b)	+ serum, 100	100	++++	—	—	—	—
"	" 20	100	0	++	++++	—	—
"	" 100	10	++++	—	—	—	—
"	" 20	10	++	++++	—	—	—
"	+ rheum, 2	100	+	++++	—	—	—
"	" 0.4	100	0	+	++++	—	—
"	" 2	10	+++	++++	—	—	—
"	" 0.2	10	+	++++	—	—	—

+, ++, etc., are proportional to the mass of growth, judged by visual comparison (see p. 269).

other inhibitory compounds [McIlwain, 1942]. Some antibacterial agents induce new nutritional needs: in such cases media adequate for normal growth of organisms but rendered unsuitable by the inhibitor are made adequate again by addition of further materials. The action of such reversing materials was in some cases shown to be due to single compounds which were related to specific processes affected by the inhibitors. Iodinin, a natural antibacterial agent of a novel type, has now been subjected to such nutritional analysis. *Streptococcus haemolyticus* has been used as test organism as it was the most sensitive of those previously studied. In a medium which was as far as possible chemically defined, $0.8-1.2 \times 10^{-6} M$ iodinin was necessary to prevent growth of streptococci for a period of 6 days; in a peptone medium, and in one containing

the most effective of the animal materials examined in cancelling the effect of iodinin, was required in quantities of about 0.2 g. to eliminate the activity of $5 \mu g.$ of iodinin.

The anti-streptococcal action of iodinin was not affected by addition of various metal salts with which higher concentrations of iodinin form lakes, nor by acid extracts of plant and animal ashes. Extracts of *Chr. iodinum* before pigmentation, and of other organisms insensitive to the inhibitor, did not antagonize its action.

Anthraquinones. The material first found to be active against iodinin in quantities small enough to be comparable with other drug-antagonists was *rheum*, which had been included in a survey of pharmaceutical materials. The activity of *rheum* was shared by other members of the groups of

anthraquinone purgatives (Table 2) which suggested that their anti-iodinin properties might be due to their therapeutically active constituents. Some of the active material in aqueous extracts of *rheum*

was removed by extraction with ether from acid but not from alkaline solutions; from the acid-ether extracts it was removed by aqueous alkalis (Table 3). This treatment also separated hydroxy-

Table 2. *Anti-iodinin activity of natural materials*

Quantity of substance (mg. or ml./ml. iodinin-containing medium (b)) needed for:—
($2 \times 10^{-6} M = 0.5 \mu\text{g./ml.}$ iodinin had been added to medium (b))

Material	Complete neutralization	Delayed or partial neutralization
Blood (human)	20, 20	4
Plasma (human)	50, 20	10, 5
Serum and plasma (horse)	100, 20	20, 4
Corpuscles	100	20
Meat (ox, hot extract)	(not by 100)	100, 50
Liver (ox, hot extract; and proprietary extract)	"	100, 100
Urine (human, 3 specimens)	(not by 100, 20, 4)	
Potato, turnip (hot extracts)	"	
Beer wort (3 specimens, used in growth of <i>Chr. iodinum</i> for pigment formation)	"	
<i>rheum</i> (hot extract, referred to original weight)	2, 2	0.4, 0.08
Aloes (hot extract, referred to original weight)	0.5	0.1
Senna pods (hot extract, referred to original weight)	5	1, 0.4
Madder (hot extract, referred to original weight)	10	2
Yeast, boiling extract	(not by 100, 40)	40, 20
Yeast, hot extract [Warburg & Christian, 1938]	"	40, 10
Yeast, plasmolysate (with acid ether)	"	20
Yeast, autolysate (marmite)	"	20, 10
Yeast, autolysate concentrates (5) active in antagonizing acriflavine inhibition [McIlwain, 1941 b]	(not by 100, 20, 4)	
<i>Escherichia coli</i> , <i>Proteus vulgaris</i> , and <i>Chr. iodinum</i> culture filtrates and hot extracts	"	

Table 3. *Properties of anti-iodinin materials in serum and rheum; activities of some concentrates and pure compounds*

Quantity of substance (molarities or mg./ml. iodinin-containing mediums (b) and (c)) needed for:—
($2 \times 10^{-6} M = 0.5 \mu\text{g./ml.}$ iodinin had been added to mediums (b) and (c))

Material	Complete neutralization	Delayed or partial neutralization
<i>rheum</i> hot extract (weights refer to original <i>rheum</i>)	2	0.4
<i>rheum</i> acid-digest (weights refer to original <i>rheum</i>)	0.4	0.2
<i>rheum</i> ether extract of acid digest, and alkaline extracts of the ether (weights refer to original <i>rheum</i>)	2	0.4
<i>rheum</i> ether extract of acid digest, and alkaline extracts of the ether (weights refer to extractives)	0.03	0.006
Aloin	0.004	0.0008
Anthraquinone; anthraquinone-2:6-disulphonate	(not by $10^{-5} M$)	
1:2-Dihydroxyanthraquinone (alizarin)	(not by $2 \times 10^{-6} M$)	
1:4-Dihydroxyanthraquinone (quinizarin)	4 to $20 \times 10^{-7} M$	$4 \times 10^{-7} M$
1:5-Dihydroxyanthraquinone (anthrarufin)	$2 \times 10^{-6} M$	$4 \times 10^{-6} M$
1:8-Dihydroxyanthraquinone (chrysazin)	$2 \times 10^{-6} M$	$4 \times 10^{-7} M$
1:2:4-Trihydroxyanthraquinone (purpurin)	Not by $10^{-5} M$	$10^{-5} M$
1:2:7-Trihydroxyanthraquinone (anthrapurpurin)	(not by $10^{-5} M$)	
Serum (ox)	20	4
Serum after extraction with light petroleum	(not by 100, 20, 4)	
Serum petroleum ether extract, with and without residue	"	
Vitamin K ₁ concentrates	(not by 0.05)	0.05
2-Methyl-1:4-naphthaquinone	0.00032; $2 \times 10^{-6} M$	2.5 to $5 \times 10^{-7} M$

anthraquinones from the drug; the crude preparation so obtained was active in quantities of from 0.006 to 0.03 mg./ml. Aloin, which consists of the almost pure glucosides of aloë-emodin (1:8-dihydroxy-3-hydroxymethylanthraquinone), was even more active (4 to 0.8 μ g. antagonizing 0.5 μ g. of iodinin). Some commercially available hydroxyanthraquinones were examined, with results recorded in Table 3; at least three were highly active (1 to 0.1 μ g. antagonizing 0.5 μ g. of iodinin).

Serum and compounds of vitamin-K activity. Anthraquinone derivatives are not known to occur in normal blood, and the properties of the iodinin-antagonizing material from this source—which is of greatest importance in relation to the possible use of iodinin—were accordingly examined. The inhibitory activity of serum was lost on digesting with acid under conditions which hydrolysed anthraquinone glucosides. Extraction with petroleum ether destroyed

antagonists, they were tested with respect to their power of neutralizing the inhibitory action of iodinin. Concentrates of vitamin K₁ (from grass, and of about 10% purity) and K₂ (from putrified fish meal) were given by Mr F. A. Robinson of Glaxo Laboratories, to whom I am much indebted. Difficulty was experienced in obtaining them in forms suitable for bacteriological testing and their activity was variable; a filtered aqueous-alcoholic solution of the K₁ preparation, at a concentration corresponding to 0.1 mg./ml. of the specimen, was however active. The water-soluble 2-methyl-1:4-naphthaquinone possessing vitamin K activity was next tested, and its activity found to be comparable to, or greater than, those of the hydroxyanthraquinones previously examined (Table 3). Table 4 gives details of the inhibitory powers of different mixtures of iodinin and the quinone. Growth of streptococci in the presence of concen-

Table 4. *Mutually neutralizing quantities of iodinin and 2-methyl-1:4-naphthaquinone*

Time (days) before appearance of visible growth in medium (b) containing:

Iodinin (<i>M</i>)	2-methylnaphthaquinone (<i>M</i>)					
	4×10^{-6}	2×10^{-6}	1×10^{-6}	5×10^{-7}	2.5×10^{-7}	1.2×10^{-7}
2×10^{-5}	$1\frac{1}{2}$	2	>7	>7	—	—
1×10^{-5}	$\frac{3}{4}$	2	3	>7	—	—
5×10^{-6}	—	$1\frac{1}{2}$	$1\frac{1}{2}$, 2	3, 7	—	—
2.5×10^{-6}	—	$\frac{3}{4}$	$\frac{3}{4}$, $1\frac{1}{4}$	$1\frac{1}{4}$, 2	>7	—
1.2×10^{-6}	—	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$, $1\frac{1}{4}$	>7	>7
6×10^{-7}	—	—	—	$\frac{3}{4}$	3	>7
3×10^{-7} and less	—	—	—	$\frac{3}{4}$	$\frac{3}{4}$	$\frac{3}{4}$

the activity, but material active alone or in combination with the extracted serum could not be recovered from the petroleum extract. Recovery was attempted by washing the solid petroleum extracts with water, alkali, or ethanol and filtering or autoclaving the extracts in order to sterilize them. Such filtration did not affect the activity of whole serum. Alkali did not extract an active material from unevaporated petroleum ether extracts, nor was extraction of the active material from serum prevented by alkali. The iodinin-antagonists in serum are thus not hydroxyanthraquinones or other phenolic or acidic compounds, but are fat soluble. Such solubility may explain the apparently limited distribution of iodinin-antagonists in natural materials, as most of these have been examined as aqueous extracts because hitherto, almost without exception, bacterial nutrients have been found to be water-soluble.

One exception to the general rule of water solubility appeared relevant to the present investigations. Woolley & McCarter [1940] showed that K-vitamins and 2-methyl-1:4-naphthaquinone increased growth of Johne's bacillus. As compounds of vitamin-K activity exist in serum and bacteria [Almquist, Pentler & Mecchi, 1938] and bear some relation to the previously examined class of iodinin-

trations of iodinin greater than the minimally effective dose, was restored by roughly proportional increases in the concentration of the naphthaquinone which, when sufficiently high, annulled the effect of a saturated solution of iodinin. Solutions of 2-methylnaphthaquinone stronger than $10^{-4}M$ were sometimes themselves inhibitory of streptococcal growth.

The relatively small difference in the active concentrations of the more potent hydroxyanthraquinones and of 2-methyl-1:4-naphthaquinone, is in contrast to their activities as anti-haemorrhagic agents. Though structural requirements for vitamin-K activity are less exacting than those for most other types of vitamin activity, and several hydroxyanthraquinones are anti-haemorrhagic [Martin & Lischer, 1941] the amounts of the latter needed by chicks are 100 times greater than that of 2-methyl-1:4-naphthaquinone, and their activities are thought by the authors to be due possibly to their breakdown products.

EXPERIMENTAL

Iodinin. A specimen of the pigment was given us by Prof. G. R. Clemo and for later work it was prepared by growth of *Chr. iodinum* by the method of Dr J. G. Davis

[1939], to whom I am indebted for practical details and a culture of the organism. The pigment was strongly adsorbed during sterilizing filtration, and sterile solutions were prepared by autoclaving weighed quantities in plugged tubes and dissolving in *N* NaOH. The pigment was kept for no more than a week or two as a *M*/100 or *M*/500 solution in *M*/5 NaOH, and dilutions for testing were prepared from this. With solutions of 10^{-4} *M* or more, iodinin separated if the alkalinity fell below that of *M*/5 NaOH, and its Na salt tended to separate from the alkaline stock solutions.

Media. (a) The amino-acid medium was Medium III of McIlwain [1940a] containing thiolacetate and pantothenate concentrates.

(b) The peptone medium was that of McIlwain, Fildes, Gladstone & Knight [1939] based on glucose-peptone, with glutamine (4×10^{-4} *M*) in place of the then unidentified factor. Oxoid peptone was the most dependable of those examined, and following the findings of p. 268 the solid peptone was extracted with light petroleum for 20 hr. in a Soxhlet apparatus before use.

(c) Before a suitable peptone was found, a medium was used consisting of (1) the amino-acids of Gladstone's [1939] medium; (2) mixture *A* of McIlwain *et al.* [1939]; (3) 0.25% of marmite, which had previously been extracted as a 10% solution, 6 times with its own volume of light petroleum, and (4) glutamine (4×10^{-4} *M*), riboflavin (10^{-7} *M*), vitamin B₆ (10^{-6} *M*) and pantothenate (10^{-7} *M*).

Organisms and conditions of growth. The test organism was either the Richards strain of *Str. haemolyticus* or a group G organism; other strains behaved similarly. About 1000 organisms/10 ml. of medium were inoculated by suitable dilution of opacity-standardized suspensions from 1-day serum-agar slopes. A typical test comprised one tube of complete medium, others to which additions of different concentrations of iodinin had been made, and still others with further additions of varying quantities of materials to be examined for iodinin-antagonizing properties. The inocula were added last, together with other addenda common to all tubes; incubation was at 37° and normally in 5% CO₂-air. Records of such an experiment are given in Table 1 in which the + signs are proportional to the mass of growth, judged at intervals, by visual comparison. In common with other inhibitors iodinin caused morphological distortion in streptococci which grew in the presence of partially inhibiting concentrations. On subculture such organisms gave normal streptococci.

Natural antagonists

Blood was collected sterilely with citrate or oxalate as anticoagulant and the growth occurring in its presence followed microscopically. When serum was separated sterilely its anti-iodinin activity was the same as that of filtered specimens. Serum fractions were sterilized by filtration. Corpuscles were washed with saline and lysed before testing.

Antraquinone purgatives. The crude extracts were prepared by twice heating the drugs at 100° with 20 parts of water. Two specimens of *rheum* were treated similarly; acid digestion was effected by refluxing 1 g. with 2 *N* H₂SO₄ (20 ml.) in N₂ for 1 hr., specimens for test being diluted, neutralized and autoclaved. Ether extraction was carried out 6 times on the acid digest with an equal volume of solvent, of which part was evaporated and part re-extracted with *N* NaOH.

Quinones. All were commercial specimens; those found active were repeatedly crystallized from aqueous ethanol. Solutions of the less soluble ones were prepared by dissolving in a little ethanol, adding this to a large volume of hot water, and were sterilized by autoclaving. Vitamin K₁ concentrates (5 mg.) were dissolved in ethanol (5 ml.), water added to about 60% (by vol.) and the solution sterilized by filtration, and washed through the filter with a little ethanol.

DISCUSSION

Modes of action of iodinin and its antagonists

The antagonistic quinones. The manner in which the quinones act was considered in relation to four known types of *in vitro* relation between antibacterial agents and substances which antagonize them, viz. (1) reaction between inhibitor and antagonist in absence of the organism [cf. Oxford, 1942]; (2) interaction which takes place only in presence of the affected organism; (3) the anti-inhibitor action of substances which promote growth under the conditions of testing [McIlwain, 1941*b*; 1942] and (4) other cases, in which it must be concluded that the antagonist is more specifically related to the process of inhibition.

Iodinin antagonism is not by mechanism (3), as streptococcal growth was optimal during testing on media (b) and (c), and similar results were obtained in three distinct media; the varied inactive addenda of Table 2 also confirm this. With respect to (1) and (2), six batches of medium (b) of final vol. 50 ml. were prepared, all of which had a concentration of 10^{-5} *M* with respect to iodinin and 2-methylnaphthaquinone. Of these, two (*A*) were inoculated and incubated normally; two (*B*) were incubated with (*A*) but not inoculated, while two (*C*) were immediately extracted 3 times with 3 ml. CHCl₃. On the second day, (*A*) and (*B*) were similarly extracted, all the extracts were dried (Na₂SO₄) and were compared colorimetrically with standard iodinin solutions. The red iodinin colours of (*B*) were 95–100% of (*C*) and of the original iodinin but (*A*) was less than 10% of these. 2-Methylnaphthaquinone was also extracted but did not obviate these conclusions.

Interaction (1) can therefore be excluded but that postulated under (2) does occur. The disappearance of pigment is probably due to reduction, for although dihydroxyphenazine or other reduction products were not isolated, phenazine and *N,N'*-dihydrophenazine were separated from a similar experiment in which phenazine di-*N*-oxide [McIlwain, 1943] was used in place of iodinin. Dihydroxyphenazine and dihydrodihydroxyphenazine derived chemically from iodinin did not affect growth of streptococci when added to the test media in concentrations of 10^{-5} to 5×10^{-4} *M*. Reduction may therefore be the mode of inactivation of iodinin; it was sometimes found to be less active anaerobically. Iodinin

($10^{-5} M$) was also changed to yellow non-inhibitory material on incubating with thiolacetate ($M/100$; but not by $M/500$) or with ascorbic acid ($M/500$; not by $M/5000$), though these interactions are not comparable with such processes as cause inactivation of iodinin by 10^{-6} to $10^{-7} M$ solutions of quinones—not themselves reducing agents—which can take place only in the presence of bacteria. Pyocyanin ($10^{-5} M$; not $2 \times 10^{-6} M$) and methylene-blue ($5 \times 10^{-6} M$; not $10^{-6} M$), however, also antagonized iodinin.

The peculiarity of the antagonistic quinones then lies (a) in their greater potency than other redox systems, (b) in the probability that compounds with vitamin-K activity are responsible for the antagonistic action of serum, and (c) in the fact that the quinones are not known to function in a manner comparable with that of e.g. methylene blue or pyocyanin in other redox systems; conversely, the extracts examined for anti-iodinin activity contained many other natural carriers, but of these the quinones proved to be outstanding as regards iodinin-antagonism.

Streptococci and other organisms growing in the presence of sub-inhibitory concentrations of iodinin destroyed it. In such an experiment with streptococci and phenazine di-*N*-oxide, phenazine was again isolated. Such destruction is, therefore, a normal behaviour of growing streptococci; the special property of the antagonistic agent is to allow this growth in the presence of higher concentrations of iodinin.

Iodinin action. That such low concentrations of iodinin are anti-streptococcal makes it probable that it interferes with specific processes in the inhibited organisms. The more potent of its antagonists are structurally related to it, and their interaction with it and the organism may be similar to that suggested for other structurally related groups of inhibitory and antagonistic agents, e.g. for various amino-sulphonic and amino-carboxylic acids [McIlwain, 1942], for related amino-acids [Gladstone, 1939], and for indole acrylate [Fildes, 1941], naphthalene acrylate [Erlenmeyer & Bloch, 1942] and tryptophan. The case of iodinin is most like the first of these instances in that the shape and polarity of the antagonist molecule is very like that of the inhibitor, except that different atoms are substituted in important groups. If the suggestions which have been made in the above instances are adopted, a working hypothesis concerning iodinin action must assume the existence of 'sites', e.g. in essential enzymes, with which iodinin forms a type of combination involving *N*-oxidic groupings. Presumably it is essential to normal growth that these sites should be involved in the reaction of e.g. the essential enzyme with compounds similar to those quinones which are known to be antagonists

of iodinin; but a sufficiently high concentration of iodinin must be able to displace the quinones and thus to inhibit growth.

The interaction between 2-methyl-1:4-naphthaquinone and iodinin is competitive within the limits of accuracy of Table 4, the quantity of iodinin necessary for complete inhibition of bacterial growth being 10 to 20 times that of the coincident naphthaquinone. Such an observation is consistent with the above hypothesis, but is not essential to it (cf. Clark, 1937). Though an observation such as this is explicable on the basis of other hypotheses, it is a phenomenon occurring in at least five of the instances quoted above. The hypothesis supposes that the quinones displace iodinin, thus allowing growth and bringing about destruction of the inhibitor; but alternatively streptococci with the aid of the quinones may change iodinin and so allow growth to be resumed. If iodinin can become attached to systems concerned with hydrogen transport in the organism, these alternatives, and those of (2) and (4) in A, above, approximate to one.

The structural relationship between iodinin and the antagonistic quinones has been used as a model in the preparation of quinoxaline di-*N*-oxides related to compounds of anti-haemorrhagic activity [McIlwain, 1943]. The di-*N*-oxides were in all cases found to be inhibitory at concentrations at which their parent quinoxalines were inactive, though these concentrations were greater than those required in the case of iodinin.

SUMMARY

1. Iodinin, the di-*N*-oxide of a dihydroxyphenazine, partly inhibited growth of streptococci in concentrations of $3 \times 10^{-7} M$ and completely inhibited it at 1.2 to $2 \times 10^{-6} M$. Phenazine di-*N*-oxide also displayed inhibitory action, but higher concentrations of it were necessary.

2. Extracts of a wide variety of plant, animal and microbial materials had relatively little influence on the inhibitory action of iodinin, but a few annulled its action and this was probably due to their containing anthraquinone and naphthaquinone derivatives. Pure hydroxyanthraquinones, and 2-methyl-1:4-naphthaquinone antagonized iodinin ($2 \times 10^{-6} M$) in concentrations of from 5×10^{-7} to $5 \times 10^{-6} M$.

3. When organisms overcame the inhibition due to iodinin and phenazine di-*N*-oxide, in the presence or absence of the quinones, the *N*-oxides were destroyed, probably by reduction. Some other hydrogen-transporting systems and reducing agents also caused destruction of iodinin and allowed growth of the organisms, but these agents were needed in higher concentrations than was necessary in the case of the antagonistic quinones.

4. As no interaction between the *N*-oxides and quinones occurred in the absence of the organisms, the quinones must have participated to some degree in the metabolism of the latter. The participation may be no more intimate than that of being concerned with hydrogen transport, but the structural similarity between the *N*-oxides and quinones sug-

gests that the two classes of compounds may function at common sites in the organism, and that the action of iodinine may be to inhibit systems normally concerned with such quinones.

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Factors Influencing the Nucleoprotein Content of Fibroblasts Growing *in vitro*

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Since Carrel [1912] first showed that the growth of tissue cultures *in vitro* could be stimulated by means of extracts of embryonic tissue, and that cultures could be maintained apparently indefinitely in such extracts, much speculation has arisen as to the chemical nature of the constituents of embryo juice responsible for the stimulation of cellular proliferation. Baker & Carrel [1926] came to the conclusion that activity lay in the protein fraction, and obtained active materials by precipitation of these with ethanol or CO₂. Rejecting the theory that embryo juice contained a specific hormone for cell proliferation, they later suggested [Carrel & Baker, 1926] that growth-activating substances are not preformed in embryo juice but are continuously made from its protein, perhaps in the cells of the cultures themselves, as the result of enzyme action. In support of this suggestion they pointed out that proteoses and higher protein degradation products have very potent growth-promoting properties, an observation which was confirmed and extended by Willmer & Kendal [1932]. Needham [1931] also rejected the view that any one particular factor was responsible for the growth-promoting properties of embryo juice, which he regarded as 'a special collo-

cation of the right nutrient substances probably protein break-down products', but later [Needham, 1942] he did not exclude the participation of 'some carrier substance of nucleotide type'. However, Fischer [1939; 1940] prepared a very active growth-promoting factor from beef embryos by isolating the nucleoproteins. The activity seemed to be located in the nucleoprotein fraction containing the ribonucleic acids, but repeated reprecipitation of the nucleoproteins resulted in loss of activity. In the active fractions the phosphorus content was higher than could be accounted for by the nucleic acid content. Some active fractions contained sulphur, probably derived from chondroitin or mucoitin sulphuric acid complexes precipitated with the nucleoproteins. The presence of ribonucleic acids in rapidly growing tissues is now well recognized [Caspersson & Schultz, 1939; 1940; Mitchell, 1940; Schultz, 1941], and the nucleoproteins of several different embryonic tissues have been shown to contain ribonucleic acids [Davidson & Waymouth, unpublished results].

By the term 'growth', applied to cells *in vitro*, as distinct from mere survival of the cells with or without cell migration and even cell division, is