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Inhibition of the Respiration of *Trypanosoma rhodesiense* by Thiols

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During an investigation of the action of sulphydryl inhibitors and reversal agents on a normal and drug-resistant strain of *Trypanosoma rhodesiense* it was found that low concentrations of certain thiols markedly inhibited trypanosome respiration. This result was unexpected in view of the earlier work of Voegtlin, Dyer & Leonard (1923), who found that cysteine and glutathione added *in vitro* in relatively high concentrations protected trypanosomes against arsenicals without exerting a toxic effect *per se* when motility was used as a criterion. In a similar type of study Chen & Geiling (1948) found that cysteine did not affect the glycolytic rate of *T. equiperdum in vitro*, and Brand, Tobie & Mehlman (1950) did not report an inhibition of trypanosome respiration by the sulphydryl compounds which they employed as protective agents against arsenicals.

However, in contrast, Strangeways (1937) observed that the motility of *T. equiperdum* was markedly inhibited when glutathione was added to the medium of Yorke, Adams & Murgatroyd (1929) in which the trypanosomes were maintained *in vitro*. This worker found that catalase abolished the inhibition and therefore concluded that it was caused by hydrogen peroxide.

We have shown that the respiratory inhibition of *T. rhodesiense* which we have observed is caused mainly by peroxide formed during the aerobic oxidation of the thiols employed.

MATERIALS AND METHODS

Biological. The strain of *T. rhodesiense* was isolated in 1923 from a patient with sleeping sickness and has subsequently been maintained by blood passage in mice. Suspensions of trypanosomes were obtained by bleeding

Table 1. *Effect of various thiols on the respiration of T. rhodesiense*

Each flask contained 3 ml. of trypanosome suspension, and 0.5 ml. of the appropriate thiol at a concentration of $7 \times 10^{-3}M$ in buffered saline was tipped in from the side arm after equilibration. Respiration was measured for 45 min. at 38°.

	BAL	Cysteine	Glutathione	Penicillamine
No. of experiments	5	6	22	6
Mean percentage inhibition	59.0	36.4	59.8	57.0
Range	54-63	15-54	8-77	34-65
Standard deviation	3.8	11.5	17.7	15.5

heavily infected rats by cardiac puncture under anaesthesia. The blood was then centrifuged at 2500 rev./min. (750 g) for 10 min. at room temperature. The supernatant was discarded and the white layer of organisms removed by Pasteur pipette and resuspended in a medium composed of 1 part of horse serum with 9 parts of phosphate-saline containing NaCl, KCl, $MgSO_4$ (Krebs & Eggleston, 1940) and 0.4% glucose. The suspension was then recentrifuged for 5 min. on a small centrifuge at 3000 rev./min. (650 g). Finally the washed trypanosomes were suspended in fresh medium in a graduated tube (0.6 cm. $\times$ 10 cm.) and again centrifuged for 5 min. at 650 g. Standard suspensions were made by adding 0.1 ml. of packed trypanosomes from the middle of the column resulting from centrifuging to each 15 ml. of medium. By this means the presence of blood cells was avoided and the number of trypanosomes kept reasonably constant. Approximately 0.3×10^8 organisms, representing a dry weight of about 0.3 mg., were present per millilitre.

Chemical. Pure commercial samples of glutathione and cysteine (Light and Co. Ltd.) were used throughout the work, and 2:3-dimercaptopropanol (BAL) was obtained from the Standards Division of this Institute. Penicillamine ($\beta\beta$ -dimethylcysteine) was a synthetic sample. All solutions were made immediately before use. Sodium pyruvate of 99% purity was prepared by the method of Lipmann (1944). Pure catalase in solution was used at a final concentration of $5 \times 10^{-8}M$.

Peroxide was estimated by modifying the colorimetric test of Fuller & Maxted (1939). A portion (3 ml.) of the peroxide solution to be estimated was added to 3 ml. of 0.1M phthalate buffer. Also, 0.1 ml. of 1% o-tolidine (w/v) in 50% ethanol and 0.05 ml. of peroxidase solution ($0.3 \times 10^{-4}M$) were added. The density of the blue colour which develops and which is stable for about 1 min. was determined with the Biochem Absorptiometer fitted with a red filter (Chance OR 2, $> 640 m\mu$). Over the range 0.009-0.09 μ mole/ml. of hydrogen peroxide the colour obeyed Beer's law and a calibration curve was constructed. Glutathione was estimated iodometrically by the method of Woodward & Fry (1932), pyruvate by the method of Goodwin & Williams (1952) and catalase in trypanosomes by the iodometric method described by Sumner & Somers (1943). The presence of keto acids was detected chromatographically (Altmann, Crook & Datta, 1951) after extraction from the medium by the method of Cavallini & Frontali (1954). When anaerobic conditions were employed conical flasks fitted with ground-glass joints and taps and containing the trypanosome suspensions were flushed for 15 min. with nitrogen freed from oxygen by the method described by Fieser & Fieser (1944); in this method the gas is passed through a solution of sodium anthraquinone- β -sulphonate in the presence of sodium dithionite and then washed in a saturated solution of lead acetate.

Table 2. *Oxygen uptake by thiols in normal medium and in that in which trypanosomes had been incubated*

Each flask contained 3 ml. of normal medium or that in which trypanosomes had been shaken for 30 min. at 38° before removal by centrifuging. Addition from the side arm was made at zero time of 0.5 ml. of $7 \times 10^{-3}M$ thiol in buffered saline. Duration of experiment, 45 min. at 38°. Mean of three experiments.

Thiol	O ₂ uptake (μ l./45 min.)	
	Normal medium	Medium in which trypanosomes had been incubated
BAL	23	35
Cysteine	22	26
Glutathione	16	26
Penicillamine	8	12

Manometric. Standard Warburg flasks of approximately 20 ml. were used for measuring gas exchanges at 38°. Standard trypanosome suspension (3 ml.) was present in the body of the flask, 0.2 ml. of 20% KOH in the centre well, and 0.5 ml. of reagents was added when required from the side arm. The gas phase in all manometric experiments was air and the oxygen uptake of the trypanosomes was linear over the experimental period of 45 min.

RESULTS

Inhibition of trypanosome respiration by thiols

When studying the reversal by $10^{-3}M$ glutathione of the inhibition of trypanosome respiration caused by $10^{-4}M$ mapharside it was found that the thiol alone frequently caused a greater inhibition than the arsenical. Cysteine, BAL and penicillamine were found to behave similarly. The magnitude of the inhibition produced by each is shown in Table 1.

The respiratory values in this table are uncorrected for the simultaneous uptake of oxygen by the thiols because it was found, as shown in Table 2, that the presence of medium in which the trypanosomes had been shaken before being removed by centrifuging increases this oxygen consumption and therefore no real control is possible. The oxygen consumption of normal medium or of that in which the trypanosomes were shaken was negligible.

Effect of anaerobiosis

At the end of the respiratory experiments the motility of trypanosomes shaken with the thiols was reduced to an extent roughly comparable with the degree of respiratory inhibition. However, when the parasites were left in contact with the thiols at 38° without shaking, their motility was but little impaired, indicating that the inhibitory action of the -SH compounds was connected with their aerobic oxidation. This view was confirmed by the following experiments. Two equal samples of a standard suspension of trypanosomes were shaken for 30 min. with 10^{-3} M thiol under aerobic and anaerobic conditions, respectively, at 38°. The suspensions were cooled in ice, and the trypanosomes centrifuged down as quickly as possible and resuspended in the same volume of fresh medium. The respiratory rate and motility of the different preparations were then compared. The mean results of three concordant experiments are shown in Table 3.

Shaking trypanosomes in the presence of thiols aerobically caused a decrease in the rate of oxygen uptake which was not reversed by resuspending the organisms in fresh medium. After anaerobic pretreatment, however, the subsequent respiratory inhibition was only 6 and 4 % in the case of cysteine

Table 3. *Effect of pre-incubation with 10^{-3} M thiols, aerobically and anaerobically, on the respiration of T. rhodesiense*

Identical suspensions of trypanosomes were shaken for 30 min. at 38° with and without thiol under aerobic and anaerobic conditions. The suspensions were then cooled in ice and the trypanosomes rapidly centrifuged and resuspended in fresh medium. The oxygen uptake of 3 ml. of each suspension was then measured.

Thiol	O ₂ uptake (μl./45 min.)			
	After anaerobic pre-incubation		After aerobic pre-incubation	
	Thiol	No thiol	Thiol	No thiol
BAL	125	146	63	153
Cysteine	129	137	79	154
Glutathione	147	153	79	161
Penicillamine	119	146	55	157

and glutathione, respectively; under similar conditions BAL and penicillamine caused a reduction of oxygen uptake of 14 and 18 %, respectively. This suggested that the reduced forms of the latter thiols were slightly toxic *per se*, although the possibility cannot be excluded that some damage occurred to trypanosomes as a result of the oxidation of thiols during the subsequent manipulations in air.

When the thiols in solution were allowed to oxidize in air until the nitroprusside test was negative, the products, either in solution or suspension at a final concentration of 10^{-3} M, did not cause inhibition of respiration.

Effect of catalase

These results suggested that a product of oxidation was responsible for the respiratory inhibition and supported the finding of Strangeways (1937) that hydrogen peroxide was involved in the inhibition of trypanosomes by glutathione. The effect of catalase on the inhibition produced by thiols was therefore tested as shown in Table 4.

The respiratory inhibition by BAL was apparently reduced when catalase was present and completely prevented in the other thiols. Boiled catalase failed to prevent the inhibition of respiration.

The addition of catalase to trypanosomes in the presence of thiols results in a slightly higher oxygen uptake than that of the control suspensions of trypanosomes without thiols. This increase appeared to be due to the oxygen used for oxidation of the thiols, the respiration of trypanosomes now being unimpaired since peroxide had been destroyed by catalase. The higher oxygen uptake of the trypanosomes in the presence of thiol when catalase was added was proved to be due to prevention of respiratory inhibition in the following experiment. Catalase in a final concentration of 5×10^{-8} M was added to 10^{-3} M glutathione in medium in which trypanosomes had been incubated for 30 min. before removal by centrifuging. No increase in oxygen uptake resulted beyond that due to oxidation of thiol. This indicated that catalase in the earlier experiments with trypanosomes in the system was preventing inhibition of trypanosome respiration caused by thiols rather than promoting

Table 4. *Effect of catalase (5×10^{-8} M) on the inhibition of T. rhodesiense respiration by thiols*

Thiol	O ₂ uptake (μl./45 min.)			
	Trypanosome suspension	Trypanosome suspension + catalase	Trypanosome suspension + thiol (10^{-3} M)	Trypanosome suspension + thiol + catalase
BAL	184	184	84	112
Cysteine	152	154	84	156
Glutathione	151	156	45	166
Penicillamine	162	160	81	179

other reactions involving oxygen uptake. Peroxidase in a concentration of $0.3 \times 10^{-4} \text{ M}$ was also able to prevent the inhibition caused by glutathione. Proom, Woiwod, Barnes & Orbell (1950) found that manganese dioxide destroyed peroxide formed in nutrient medium, thus preventing inhibition of growth of *Shigella dysenteriae*. However, 0.1% manganese dioxide, previously washed in Krebs saline, did not prevent inhibition of trypanosome respiration by 10^{-3} M glutathione. Palladized charcoal (Mozingo, 1946) and Raney nickel (Mozingo, 1941), under the same conditions, also failed to prevent respiratory inhibition, although none of these substances themselves caused inhibition.

Peroxide formation during thiol oxidation

The test for peroxide of Fuller & Maxted (1939) was used to determine whether peroxide was formed as a result of trypanosome metabolism in the presence of added thiols or directly from the aerobic oxidation of thiols. The amounts of peroxide detected when thiols were shaken in the Warburg apparatus in air at a concentration of 10^{-3} M are shown in Table 5.

The results given establish that peroxide formation occurs under the conditions described with all four thiols. It also occurred to a lesser extent when thiols were shaken with buffered saline alone. The observed rate of production varies widely from one thiol to another. However, it is not certain that the values shown accurately represent the amount of peroxide formed, for we observed that the presence of much unoxidized thiol interferes with the production of colour. Randall (1946) has pointed out that peroxidase (which is used in the estimation) catalyses the oxidation of reduced thiols by peroxide, and that reduced thiols can decolorize the product formed in the peroxide test.

Webb & van Heyningen (1947) have observed that BAL inhibits catalase in a concentration four times that which we have employed. However, when BAL was added to medium containing $5 \times 10^{-8} \text{ M}$ catalase and shaken in flasks at 38° peroxide could not be detected, although it was detectable in relatively large amounts in the absence of catalase. This suggests that the failure of catalase to prevent completely the inhibition of trypanosome respiration by BAL, as shown in Table 4, is due to the fact that BAL itself is toxic in some degree and not to the inactivation of catalase by this thiol.

Inhibition of trypanosome respiration by hydrogen peroxide

The effectiveness of hydrogen peroxide as an inhibitor of trypanosome respiration is shown in Table 6. Comparison of stabilized and unstabilized hydrogen peroxide showed that the stabilizer had

Table 5. *Production of peroxide during shaking of thiols in air at 38°*

Usual medium (3 ml.) in a Warburg flask was mixed with 0.5 ml. of $7 \times 10^{-3} \text{ M}$ thiol at zero time and samples were removed at intervals for estimation of peroxide.

Time (min.)	$\mu\text{moles of peroxide in reaction vessel}$			
	BAL	Cysteine	Glutathione	Penicillamine
0	0	0	0	0
15	0.19	0.22	0.06	0.02
30	1.16	0.26	0.09	0.44
45	0.88	0.25	0.11	0.42
75	0.88	0.19	0.002	0.36

Table 6. *Inhibition of T. rhodesiense respiration by hydrogen peroxide*

Trypanosome suspension (3 ml.) was placed in the flask and 0.5 ml. of hydrogen peroxide in saline was placed in the side arm.

Hydrogen peroxide (μmoles)	5	1	0.2	0.04	0.02
Percentage inhibition after 45 min.	81	51	28	2	7

no effect on trypanosome respiration in the concentration present.

It is seen from these results that trypanosome respiration is inhibited by concentrations of peroxide of the same order as those detected during oxidation of the thiols (Table 5).

In order to find whether or not other respiratory inhibitors were being formed as a result of the interaction of the peroxide produced from glutathione with other components of the system, trypanosome suspensions were shaken for 30 min. in the presence and absence of 10^{-3} M glutathione. The trypanosomes were removed by centrifuging and catalase in the concentration previously used was added to destroy any peroxide formed. The respiratory rate of fresh trypanosomes was immediately compared in the two media. No significant difference was found, indicating that peroxide itself is the toxic factor involved.

Peroxide destruction in the presence of trypanosomes

In contrast to the regular appearance of peroxide on shaking thiols in air in the absence of trypanosomes, only a minute amount was detected in their presence. This trace, which was observed early in the experiment, rapidly disappeared. When $1 \mu\text{mole}$ of peroxide was added to 3 ml. of respiring trypanosome suspension the peroxide concentration fell until none was detectable after 20 min., although in the absence of trypanosomes it persisted well beyond the experimental period.

We therefore examined washed whole organisms and homogenates, prepared by mechanical disinte-

gration in water, for the presence of catalase. Particular care was taken to avoid the presence of red cells by repeated washings. Negligible catalase activity was detected [Kat. $f = 0.2$].

In the absence of evidence for the presence of catalase it was concluded that trypanosome metabolic products were destroying peroxide. To demonstrate this samples of a trypanosome suspension respiring under the usual conditions were taken at 10 min. intervals and freed from trypanosomes by centrifuging. Peroxide was added to each sample to give a concentration of $1 \mu\text{mole}$ in 10 ml., and after incubation for 5 min. at 38° the peroxide was estimated. In a sample of medium which had not been in contact with trypanosomes no destruction of peroxide occurred during the 5 min. incubation. However, in the other samples the power to destroy peroxide increased with the length of the respiration period and, therefore, with the amount of metabolites present. It is known that *T. rhodesiense* produces large amounts of pyruvate (Fulton & Stevens, 1945), and this was the only keto acid identifiable by paper chromatography among the products of metabolism in our system. Analysis showed that $2\text{--}5 \mu\text{moles}$ were present in each Warburg vessel at the end of the usual equilibration period. This concentration increased to about $12 \mu\text{moles}$ after 45 min. under normal conditions.

It is well known that peroxide reacts with pyruvate as follows:

On adding $0.25 \mu\text{mole}$ of peroxide to $10 \mu\text{moles}$ of pyruvate in saline at 38° we found that peroxide was not detectable after 5 min. Therefore, more than enough pyruvate is formed by the trypanosomes during the equilibration period in the Warburg apparatus to destroy the small amount of hydrogen peroxide detected during the oxidation of the thiols in the absence of pyruvate. However, even when $10 \mu\text{moles}$ of pyruvate were added at the beginning of the experiment, before addition of the thiol, inhibition of respiration was not prevented except in the case of glutathione.

When $1 \mu\text{mole}$ of hydrogen peroxide was added from the side arm at 10 min. intervals to each of a series of flasks containing trypanosome suspensions, the resulting respiratory inhibition decreased markedly as more pyruvate appeared in the system and reacted with the peroxide formed.

Rate of oxidation of glutathione

Since the object of adding glutathione to suspensions of trypanosomes was to protect them against the action by arsenicals, it was important to determine the rate at which $-\text{SH}$ groups were inactivated in the system.

Table 7. *Rate of disappearance of $-\text{SH}$ groups in glutathione in the presence of trypanosomes, added pyruvate and catalase*

Glutathione was added to each flask to give a final concentration of 10^{-3}M , and $-\text{SH}$ groups were estimated by iodine titration at intervals.

Time (min.)	μmoles of reduced glutathione			
	Medium alone	Medium + trypano- somes	Medium + pyruvate $4 \times 10^{-3}\text{M}$	Medium + catalase $5 \times 10^{-8}\text{M}$
0	3.15	3.15	3.15	3.15
15	2.02	2.65	2.23	2.41
30	1.39	2.37	1.82	1.83
45	0.9	2.23	1.18	1.77

From the results in Table 7 it is seen that the oxidation of glutathione was slower in the presence of trypanosomes and also of added pyruvate. Catalase also reduced the rate of oxidation. It is probable that pyruvate, like catalase, retards the oxidation of $-\text{SH}$ groups by destroying peroxide.

In some 20% of the experiments performed with glutathione there was no inhibition of trypanosome respiration under the usual conditions, although the other thiols always gave rise to inhibition. Peroxide was not detected on these occasions when glutathione was shaken in medium without trypanosomes, and iodine titration revealed a marked decrease in the rate of oxidation of $-\text{SH}$ groups. By eliminating the different components of the medium in turn on one such occasion it was found that phosphate in the buffer interfered with glutathione oxidation. This suppression was not overcome by extraction of the phosphate buffer with 8-hydroxyquinoline, to remove heavy metals which might have destroyed peroxide. It was also found that some specimens of glutathione oxidized more readily than others, and on one occasion analysis showed that copper was present in minute amounts in the sample producing inhibition, but was absent in a second sample which did not cause inhibition. It has long been established that copper catalyses the oxidation of glutathione (Voegtlin, Johnson & Rosenthal, 1931).

DISCUSSION

It has been established in these experiments that peroxide formed during the oxidation of thiols is responsible for the observed inhibition of *T. rhodesiense* respiration. The partial or complete prevention of the inhibition by catalase in all our experiments strongly suggests that hydrogen peroxide, rather than an organic peroxide, is involved. The fact that earlier workers failed to observe a toxic action of thiols on trypanosomes can readily be explained by their use of a whole-blood

medium in which the catalase present would destroy any peroxide formed, or because they did not subject the thiols to conditions in which oxidation could readily take place.

Thurlow (1925) found that nitrite was oxidized to nitrate in the presence of cysteine or glutathione and peroxidase. It was therefore suggested that peroxide was formed as a result of thiol autoxidation. Holtz & Triem (1937) and later Schales (1938), using a very sensitive luminescent test, observed that peroxide formation occurred during autoxidation of a number of substances including cysteine and glutathione. However, it has not been suggested that peroxide formation is involved in the observed inhibition by thiols of the respiration of yeast (Quastel & Wheatley, 1932; Runnström & Sperber, 1938), and of bacteria (Cook, Perisutti & Walsh, 1946) or of the motility of spermatozoa (MacLeod, 1951; White, 1954, 1955). In these instances aerobic conditions suitable for the oxidation of thiols and peroxide formation were employed. Tosić (1947) has shown that bull spermatozoa are very sensitive to hydrogen peroxide produced during the metabolism of certain amino acids.

Evidence for the production of hydrogen peroxide during oxidation of BAL was given by Slater (1949), but he concluded that it was not the main cause of inhibition of the mammalian succinoxidase system by BAL. In our system it appeared that peroxide production played a major part in the toxicity of BAL since the inhibition was largely prevented by catalase.

Our results show that the respiration of *T. rhodesiense* is markedly inhibited by low concentrations of hydrogen peroxide comparable with those detected during oxidation of the thiols. Reiner & Leonard (1932) and Strangeways (1937) found that peroxide also affects the motility of *T. equiperdum*. Little or no catalase has been detected in trypanosomes (Krijgsman, 1936; Strangeways, 1937; Harvey, 1949), and we failed to detect catalase activity in *T. rhodesiense* when care was taken to exclude all red cells. Their presence may account for the catalase activity of very low order claimed by workers with other species of trypanosomes. In view of the absence of catalase it is not surprising that trypanosomes are affected by low concentrations of peroxide.

There is some correlation between the effectiveness of thiols as inhibitors of trypanosome respiration and the amount of peroxide which each produces. However, the inhibition caused by glutathione is relatively greater than is indicated by the amount of peroxide detected, which is less than that detected with the other thiols. Even when an approximate allowance is made for the oxygen consumed during the autoxidation of the thiols in

the system, glutathione is still found to cause a greater inhibition, on average, than either cysteine or penicillamine. This problem requires further investigation.

Because of the ability of pyruvate to react with hydrogen peroxide its production by trypanosomes was a complicating factor in our experiments. Not only does its production make the estimation of peroxide from thiol oxidation in the presence of trypanosomes impossible but, as Slater (1952) has found, it causes an increase in the oxygen consumption during thiol oxidation through its reaction with the peroxide produced. As the amount of pyruvate in the system is continuously increasing an accurate correction for the amount of oxygen taken up, other than by trypanosomes, is rendered difficult when calculating respiratory inhibitions.

It appears that although the pyruvate formed by trypanosomes destroys peroxide produced from the thiols, it fails to prevent the inhibition of respiration. This may be due to the comparatively slow rate of reaction of peroxide with pyruvate in our system. It is not clear why excess pyruvate offers greater protection against added hydrogen peroxide than against the apparently lower concentration produced during the oxidation of thiols. It is possible that the slow and continuous production of peroxide by this means is more effective as an inhibitor. In a somewhat analogous way peroxide generated within a system has been found to be more reactive as a substrate for demonstrating the peroxidative activity of catalase than added peroxide (Keilin & Hartree, 1945).

In conclusion it appears that thiols such as cysteine, glutathione and penicillamine, but not BAL, may still be used in experiments on the reversal of inhibition of trypanosome respiration by drugs. Under our conditions, in which serum replaces whole blood, the addition of catalase in appropriate concentration overcomes the toxic effect of the peroxide formed.

SUMMARY

1. 2:3-Dimercaptopropanol (BAL), cysteine, glutathione and penicillamine at a concentration of $10^{-3}M$ caused inhibition of the respiration and motility of *Trypanosoma rhodesiense* *in vitro*.
2. Catalase partly prevented the inhibition by BAL and completely prevented that caused by the other thiols.
3. Under anaerobic conditions the toxicity of BAL and penicillamine was greatly reduced, and that of cysteine and glutathione completely eliminated.
4. Catalase could not be detected in the trypanosomes, but peroxide, whether added to or formed in

the system, was destroyed in the presence of the respiring organisms by reacting with the pyruvate which they produced.

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Studies in Carotenogenesis

22. THE STRUCTURE OF ECHINENONE*

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The carotenoid echinenone was first obtained by Lederer (1935, 1938) from the gonads of the sea-urchin *Paracentrotus lividus*; it has now been shown to be widely distributed in marine invertebrates (see, for example, Goodwin, 1952). Lederer concluded that echinenone was a monoketo derivative of β -carotene, and Goodwin & Taha (1950) concluded, from spectrographic data on echinenone and the hydroxy- β -carotene derived from it by the action of aluminium isopropoxide, that it was probably 4-oxo- β -carotene. The present paper reports the application of new developments in carotenoid chemistry to this problem, which has made it possible to provide detailed confirmation of the proposed structure.

Goodwin & Taha (1950) concluded that the presence of a conjugated keto group in echinenone was strongly indicated by (a) the damping down of the fine structure of its absorption spectrum, (b) the shift of the main maximum (in light petroleum) from 448–449 m μ . in β -carotene to 453–454 m μ . in echinenone [453–454 m μ . is a more accurate value for λ_{max} . than 458 m μ . previously recorded by Goodwin & Taha (1950)], and (c) the production of a compound with an absorption spectrum indistinguishable from that of either β -carotene or cryptoxanthin (3-hydroxy- β -carotene) on reduction of the keto group of echinenone to a hydroxyl group. As cryptoxanthin and reduced echinenone were not separable chromatographically, it was suggested that the two were identical and that cryptoxanthin was 4-hydroxy- β -carotene. Karrer

* Part 21: van Niel, Goodwin & Sissins (1956).