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The Effect of Stress and Corticotrophin on the Concentrations of Vitamin C in Blood and Tissues of the Rat

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When the pituitary–adrenal axis is stimulated the concentration of vitamin C in the blood is increased (Long, 1947; Allison, 1955; cf. Lloyd & Sinclair, 1953). Corticotrophin has been reported to increase the vitamin C concentration in human plasma (Stewart, Horn & Robson, 1953) and its urinary excretion in man and guinea pigs (Prunty, Clayton, McSwiney & Mills, 1955); rats exposed to cold show increased urinary excretion of vitamin C (Monier & Weiss, 1952).

The present paper reports work on the distribution and nature of vitamin C in the blood and tissues of anaesthetized rats subjected to various procedures. The first batch of 15 rats investigated included the 13 used by Lahiri & Lloyd (1962), further estimations of the concentration of vitamin C in the blood being made after the removal of the second adrenal. It has been found that, when the right adrenal is excised and the left adrenal excluded from the circulation or excised, the vitamin C concentration in the arterial blood rises during stress, and the vitamin C concentration of blood collected at the end of the experiment from the inferior vena cava at a point above the diaphragm exceeds that in arterial blood by some 70%. In a second batch of rats the concentration of vitamin C in the arterial blood rose more than that in the femoral venous blood, but it did not exceed the concentration in the inferior vena cava blood. The concentrations of vitamin C in various tissues have been measured in a third batch of rats subjected to four different procedures after anaesthesia; this has led to the conclusion that the increase in the concentration of vitamin C in blood is not accompanied by a depletion of the tissues examined. Most of the experiments have involved parallel estimations of ascorbate and of ascorbate plus dehydroascorbate plus dioxogulonate. The difference between these estimates represents dehydroascorbate plus dioxogulonate.

EXPERIMENTAL

Materials

These were the same as those used by Lahiri & Lloyd (1962), with the exception that the corticotrophin administered to the second group of rats in the third batch was hog corticotrophin (Cortotrophin, Organon Laboratories Ltd.), 25 i.u. being dissolved in 1 ml. of sterile 0·9% (w/v) NaCl for injection.

Methods

The methods used by Lahiri & Lloyd (1962) were applied to blood and adrenals without modification (all values for ascorbate, dehydroascorbate and dioxogulonate are expressed in terms of ascorbic acid). Other tissue concentrations were measured in the third batch of rats: the animals were decapitated, blood was collected in oxalate-treated tubes, and the tissues were dissected out, cleaned of unwanted tissues, freed from adhering blood with filter paper and immediately frozen in solid carbon dioxide. The tip of the left lobe of the liver, the pancreatic end of the spleen, a portion of the lower lobe of the left lung, a portion of the frontal lobe of brain, the left kidney, both adrenals and the pituitary were collected from each animal. The whole liver was taken from some animals for weighing. Small pieces of tissue were dropped into weighed tubes containing appropriate amounts of 32% (w/v) metaphosphoric acid. All tissues, except pituitary, were then ground in a mortar with acid-purified sand (40–100 mesh, British Drug Houses Ltd.) and made up to a definite volume with water. The pituitary was homogenized in a small glass homogenizer; 0·5 ml. of 32% metaphosphoric acid and 3·0 ml. of water were used for each pituitary. The total volume of the homogenate was obtained from the weight of the tissues and the volume of acid and water added. The final percentages (w/v) of tissue in the acid homogenates were approximately: liver, 2·4; spleen, 1·2; lung, 2·3; brain, 1·5; kidney, 4; adrenals, 0·20; pituitary, 0·22. The supernatant after centrifuging was then used for the estimations.

Animals

The first batch has been described by Lahiri & Lloyd (1962); the second batch was similar. The third batch was also similar except that the rats weighed about 200 g. All three batches had the same diet.

General procedures

First batch. As described by Lahiri & Lloyd (1962) samples of mixed hepatic venous blood were collected from the inferior vena cava above the diaphragm about 1 hr. after laparotomy and the exclusion of the adrenals from the circulation. Ascorbate and ascorbate plus dehydroascorbate plus dioxogulonate were estimated in these samples and in samples drawn simultaneously from the carotid artery.

Second batch. Blood samples were collected at selected time-intervals from the carotid artery and the femoral vein

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Table 1. *Effect of laparotomy, effective adrenalectomy and corticotrophin treatment on the concentration of vitamin C in the blood of anaesthetized cortisone-treated rats (first batch)*

Experimental details are given in the text. The results are given as means ± S.E. Expt. A: arterial blood immediately after laparotomy and about 1 hr. later (15 rats). Expt. B: comparison of arterial blood and blood from the inferior vena cava above the diaphragm about 1 hr. after laparotomy (9 rats out of the above 15).

Expt. A

Initial			Final		
Ascorbate + dehydroascorbate + dioxogulonate	Ascorbate	Difference	Ascorbate + dehydroascorbate + dioxogulonate	Ascorbate	Difference
1·07 ± 0·10	1·02 ± 0·07	0·05 ± 0·026	1·92 ± 0·12	1·86 ± 0·13	0·06 ± 0·033

Expt. B

Arterial			Inferior vena cava		
Ascorbate + dehydroascorbate + dioxogulonate	Ascorbate	Difference	Ascorbate + dehydroascorbate + dioxogulonate	Ascorbate	Difference
1·70 ± 0·13	1·64 ± 0·15	0·06 ± 0·008	3·03 ± 0·23	2·72 ± 0·26	0·31 ± 0·059

of six laparotomized heparinized rats under Nembutal anaesthesia. At the end of the experiment (about 100 min. after laparotomy) a sample was collected from the inferior vena cava above the diaphragm. The right adrenal gland had been excised soon after laparotomy, and the left adrenal gland was excised at the end of the experiment.

Third batch. These animals were divided into four groups. They were given water but starved for about 18 hr. before being anaesthetized. The six rats in group I were given 60 mg. of Nembutal/kg. body wt. and then decapitated, blood being collected from the neck.

The nine rats in group II were anaesthetized with 42 mg. of Nembutal/kg. and then given 40 i.u. of corticotrophin/kg. intraperitoneally. After 55 min. a further dose of Nembutal (60 mg./kg.) was given, and the animals were decapitated after a further 5 min. The anaesthetized animals were kept in an incubator at 32°, the oral temperature being taken with a clinical thermometer at the beginning and end of the experimental period.

The six rats in group III were given 1·6 ml. of 0·9% NaCl/kg. instead of corticotrophin, and were otherwise treated like the rats of group II.

The eight rats in group IV were laparotomized after anaesthesia and blood equal to 2% of the body weight was allowed to leave through a cannulated carotid artery. They were otherwise treated like the rats of groups II and III, except that no injection was given, and ascorbate plus dehydroascorbate plus dioxogulonate and ascorbate alone were estimated in the arterial blood collected after laparotomy and in the neck blood obtained after decapitation.

RESULTS

First batch of rats. Table 1 summarizes the results, and the individual values by both methods for the differences between the final and initial arterial bloods and between the final arterial blood and the blood from the inferior vena cava above the diaphragm (this includes hepatic venous blood) are plotted in Figs. 1 and 2. The differences between

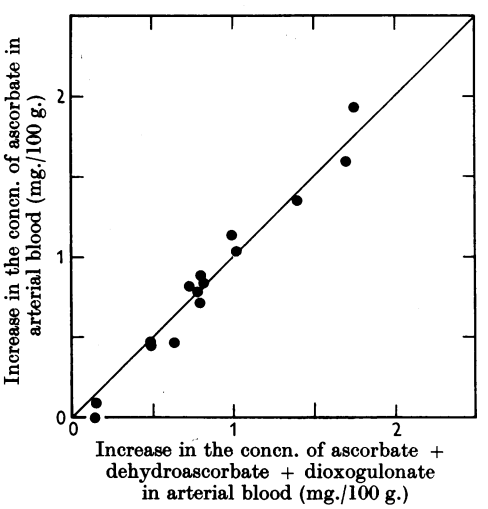

Fig. 1. Nature of the increased concentration of ascorbate plus dehydroascorbate plus dioxogulonate in the arterial blood of rats treated with cortisone, anaesthetized, laparotomized, treated with corticotrophin and in effect adrenalectomized. The increase in ascorbate is plotted against the corresponding increase in ascorbate plus dehydroascorbate plus dioxogulonate. The relation of the points to the line of equality shows that virtually all the increase is in ascorbate.

the two sets of arterial bloods and the two final bloods (arterial and inferior vena cava) are highly significant.

Of the estimates of dehydroascorbate plus dioxogulonate, which are much smaller, only those for initial arterial blood (0·054 mg./100 g.; 15 rats; $P < 0·05$) and for inferior vena cava blood (0·31 mg./100 g.; 9 rats; $P < 0·01$) are statistically significant. The latter concentration may be physiologically important. Fig. 1 shows that the increases

in the ascorbate plus dehydroascorbate plus dioxogulonate are entirely accounted for by the increases in ascorbate in arterial blood, but Fig. 2 shows that on the average the excess of ascorbate plus dehydroascorbate plus dioxogulonate in inferior vena cava blood (concentration in inferior vena cava − final concentration in arterial blood) is 0·25 mg./100 g. greater than the corresponding excess of ascorbate. This result, which is largely due to the dehydroascorbate plus dioxogulonate in inferior vena cava blood, should be compared with the results (Table 2) for the six rats in the second batch: under slightly different conditions the difference (0·06 mg./100 g.) between the final concentrations of ascorbate plus dehydroascorbate plus dioxogulonate and ascorbate alone in the inferior vena cava is not statistically significant. Combination of the two sets of values for inferior vena cava blood gives a mean concentration of dehydroascorbate plus dioxogulonate of 0·207 mg./100 g. (S.E. 0·056), and this is significantly different from zero ($P < 0·01$).

Second batch of rats. The mean concentration of ascorbate was 265 mg./100 g. in the right adrenal, removed soon after laparotomy, and 246 mg./100 g. in the left adrenal, removed some 90 min. later after haemorrhage. The low initial concentration and the small difference implies that the right adrenal had lost some ascorbate before excision. The time-course of the changes in the concentration of ascorbate in arterial and femoral-vein blood is shown in Fig. 3. The mean arteriovenous difference is significantly different from zero ($P < 0·05$) at 90 min. The mean difference between inferior vena cava blood and the final arterial blood is obviously significantly different from zero. The initial and final concentrations in venous blood are summarized in Table 2.

Third batch of rats. The average loss of weight of a rat after the 18 hr. period without food was about 14 g. in all groups. The body temperatures lay between 36° and 38·6°, those of corticotrophin-treated rats being in the higher part of this range. Mean liver weights, expressed as percentages of body weights, were: group I, 3·2; group II, 3·3; group III, 3·1; group IV, 3·3. Harris and his co-workers found an increase in liver weight in guinea pigs and rabbits after administration of corticotrophin for a long period (Harris, 1953; Constable, Harris & Hughes, 1956).

Table 3 summarizes the results of estimating the concentrations of ascorbate plus dehydroascorbate plus dioxogulonate and of ascorbate alone in tissues from the four groups of rats in this batch.

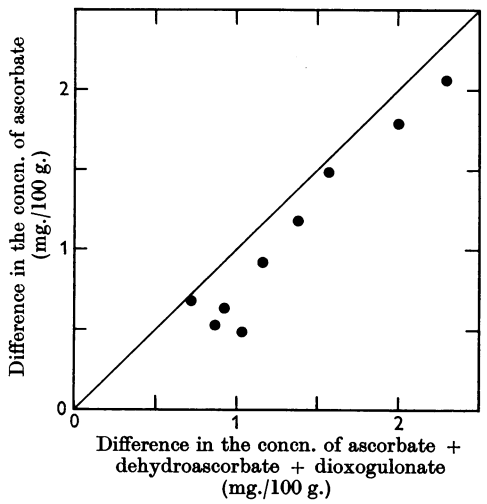

Fig. 2. Nature of the difference in concentration of ascorbate plus dehydroascorbate plus dioxogulonate between arterial blood and blood from the inferior vena cava above the diaphragm in rats stressed as described in Fig. 1. The corresponding difference in the concentrations of ascorbate is plotted against the difference in the concentrations of ascorbate plus dehydroascorbate plus dioxogulonate. The relation of the points to the line of equality shows that the difference in the concentrations of ascorbate plus dehydroascorbate plus dioxogulonate is not solely attributable to ascorbate.

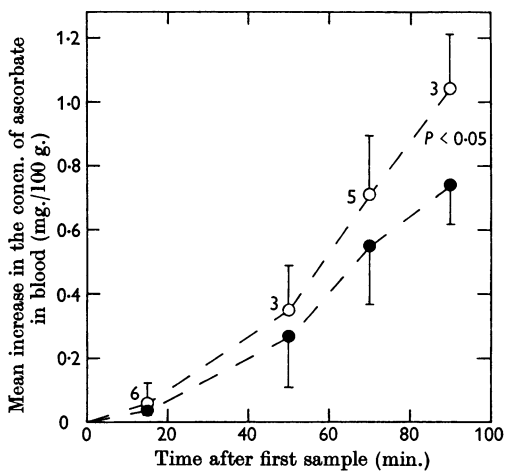

Fig. 3. Time-course of increases of the concentration of ascorbate in the blood of laparotomized anaesthetized rats. ○, Mean difference between the arterial blood and the corresponding initial femoral-vein blood; ●, mean difference between the femoral-vein blood and the corresponding initial femoral-vein blood. Vertical lines represent S.E. The numbers by the points indicate the numbers of rats used for calculating the mean increases at any one time. The total number of rats involved was six. After 95–100 min. the mean difference between the concentrations in the blood from the inferior vena cava and in the corresponding initial femoral-vein blood was 1·92 mg./100 g. (S.E. 0·19).

Table 2. *Concentrations of ascorbate and of ascorbate plus dehydroascorbate plus dioxogulonate in venous blood of anaesthetized rats after laparotomy (second batch)*

Experimental details are given in the text. The results are given as means ± S.E. (six rats were used). The initial values refer to blood taken immediately after laparotomy and the final values to blood taken 95 min. later.

	Final Blood from inferior vena cava above diaphragm		
Initial femoral-vein blood ascorbate	Ascorbate + dehydro-ascorbate + dioxogulonate	Ascorbate	Difference
1·06 ± 0·11	3·04 ± 0·24	2·98 ± 0·21	0·06 ± 0·08

Table 3. *Concentrations of ascorbate and of ascorbate plus dehydroascorbate plus dioxogulonate in tissues of anaesthetized rats after various treatments (third batch)*

Experimental details are given in the text. The results are given as means ± S.E. The anaesthetized animals were killed: (group I, 6 rats) at the beginning of anaesthesia; (group II, 9 rats) about 1 hr. after the administration of corticotrophin; (group III, 6 rats) about 1 hr. after the administration of 0·9% NaCl; (group IV, 8 rats) about 1 hr. after laparotomy and haemorrhage.

Organ	Ascorbate + dehydro-ascorbate + dioxogulonate	Ascorbate	Difference	Ascorbate + dehydro-ascorbate + dioxogulonate	Ascorbate	Difference
	Group I			Group II		
Liver	27·6 ± 1·3	23·7 ± 1·2	3·9	35·3 ± 1·7	34·9 ± 1·6	0·4 ± 0·7
Spleen	36·7 ± 2·2	30·0 ± 2·4	6·7	40·0 ± 1·9	34·9 ± 2·3	5·1 ± 1·2
Lung	29·4 ± 2·3	20·0 ± 3·5	9·4	29·6 ± 2·2	19·8 ± 2·0	9·8 ± 1·2
Brain	43·4 ± 0·4	40·0 ± 0·4	3·4	43·9 ± 1·7	42·0 ± 1·3	1·9 ± 0·8
Kidney	16·5 ± 1·1	13·8 ± 1·1	2·7	17·7 ± 1·2	17·1 ± 1·1	0·6 ± 0·3
Adrenal	386 ± 12	356 ± 14	30	254 ± 10	244 ± 10	10 ± 8
Pituitary	116 ± 6	102 ± 6	14	131 ± 7	116 ± 5	15 ± 6
Blood from neck	0·85 ± 0·07	0·87 ± 0·09	−0·02	1·10 ± 0·07	1·12 ± 0·08	−0·02 ± 0·02
	Group III			Group IV		
Liver	27·2 ± 1·7	21·6 ± 1·0	5·6 ± 1·5	24·1 ± 1·6	21·7 ± 1·6	2·4
Spleen	34·4 ± 1·9	24·5 ± 1·6	9·9 ± 2·4	36·4 ± 1·8	29·4 ± 1·4	7·0
Lung	32·1 ± 1·7	22·9 ± 1·9	9·2 ± 1·1	33·8 ± 1·0	25·6 ± 1·6	8·2
Brain	42·4 ± 0·6	38·6 ± 1·1	3·8 ± 0·6	42·6 ± 1·1	40·1 ± 0·4	2·5
Kidney	15·2 ± 1·0	12·0 ± 0·6	3·2 ± 1·4	15·5 ± 1·2	13·4 ± 1·0	2·1
Adrenal	346 ± 15	326 ± 20	20 ± 7	257 ± 15	239 ± 19	18
Pituitary	116 ± 6	99 ± 6	17 ± 4	113 ± 3	100 ± 7	13
Blood from neck	0·78 ± 0·09	0·72 ± 0·05	0·06 ± 0·03	1·51 ± 0·12	1·45 ± 0·11	0·06
Blood from carotid artery (after laparotomy)	—	—	—	0·77 ± 0·06	0·81 ± 0·08	−0·04

The results for the four groups are fairly similar, with the corticotrophin-treated group II showing higher ascorbate concentrations in liver, spleen, kidney, brain, blood and pituitary than the saline-treated group III ($P < 0·001$, $< 0·01$, $< 0·01$, $< 0·1$, $< 0·01$, and $< 0·1$ respectively). The adrenal content is, on the other hand, lower. There are also similar though in general smaller differences between group IV (laparotomy and haemorrhage) and group III. The mean rise in the concentration in blood associated with laparotomy is statistically significant (0·64 mg./100 g.; S.E. 0·095; $P < 0·01$).

The individual estimates of dehydroascorbate plus dioxogulonate are also summarized in Table 3. Many of the means of the individual values are statistically significantly different from zero. They tend to imply the presence of a real quantity of dehydroascorbate or dioxogulonate or both in the tissue extract, but it must be remembered that there may be interference from substances unrelated to vitamin C and that ascorbate is highly susceptible to oxidation during protein precipitation; losses are inevitable if some oxygenated haemoglobin is present. Blood lowers ascorbate

concentrations by oxidation in this way and also, because its own content of ascorbate is low (and indeed is estimated as zero after precipitation by the usual methods in the presence of oxygen), by simple dilution of tissue concentrations. Apart from the small organs (adrenals and pituitary, where high concentrations go with relatively large surfaces), the brain appears to contain least blood and the lung most, and this correlates with the estimates of dehydroascorbate plus dioxogulonate. But though the absolute values obtained for dehydroascorbate plus dioxogulonate must be viewed with caution, comparison of the differences in Table 3 seems to show that there is less dehydroascorbate plus dioxogulonate in liver, spleen, brain, kidney and adrenals from rats treated with corticotrophin than in those treated with 0·9 % sodium chloride; only the liver difference, however, is statistically significant ($P < 0·05$).

DISCUSSION

The experiments on all three batches of rats used in this work show that anaesthetized rats subjected to laparotomy have, after about 1 hr., increased concentrations of ascorbate in blood, whether or not corticotrophin is given, and whether or not the adrenal is contributing to the circulation. The blood from the inferior vena cava above the diaphragm, i.e. above the point at which hepatic venous blood flows in, has a higher concentration of ascorbate than the arterial blood (first and second batches); the femoral venous blood contains less (second batch). Thus the increased concentrations in the blood appear to come from visceral sources and not from the tissues of the leg.

The measurements on the third batch of rats show that, though the concentrations in the blood rise in rats treated with corticotrophin (group II), and in those subjected to laparotomy and haemorrhage (group IV), the concentrations in tissues either remain essentially constant or rise significantly, as in liver, spleen and kidney in the corticotrophin-treated animals. This was an unexpected finding, though Sayers, Sayers, Liang & Long (1945) recorded that, after haemorrhage of about 2 ml./100 g. body wt., the concentrations of ascorbate in liver and plasma increased in normal and hypophysectomized rats.

The extra quantity of ascorbate in the blood is absolutely fairly small, but the progressively increasing arteriovenous difference for the leg (Fig. 3), the difference between the concentrations in the blood of the inferior vena cava and of the femoral vein, and the general increase (lungs and adrenals excepted) in the concentrations in the tissues of the corticotrophin-treated animals and laparotomized animals, make it unlikely that the blood increases are due to a depletion of tissue contents: they point, nevertheless, to an increased abdominal contribution of vitamin C to the blood, and the rise in the concentration in the liver points to that organ as its source in the corticotrophin-treated animal.

The animals subjected to laparotomy and bleeding (group IV) show concentrations of ascorbate in tissues equal to or greater than those of animals treated with 0·9 % sodium chloride, except for that in the adrenals. The total adrenal difference in content is about 50 μg. per animal: the increase in the ascorbate content of blood is about 70 μg., and this corresponds to a total extracellular-fluid increase of some 300 μg. The difference in the content in the liver of ascorbate plus dehydroascorbate plus dioxogulonate between animals treated with corticotrophin and with 0·9 % sodium chloride is about 400 μg., which is greatly in excess of the difference in the adrenal contents.

The estimates of dehydroascorbate plus dioxogulonate in animals treated with 0·9 % sodium chloride are, with the exception of the lung, greater than those in corticotrophin-treated animals, and, to a smaller extent and with the exception of blood, than those in animals subjected to laparotomy and haemorrhage. The individual estimates are small and, with the exception of liver in corticotrophin-treated animals, not statistically significant when considered in isolation, but the overall effect seems worthy of note.

The overall picture is that there is an increase in the amount of ascorbate in blood and tissues (adrenals and lungs excepted) in the corticotrophin-treated and, to a smaller extent, in the laparotomized rats: this is accompanied by a general decrease in the amount of dehydroascorbate plus dioxogulonate. This latter decrease is probably not entirely attributable to analytical difficulties related to the blood content of tissues, and the values for liver weights support the view that the absolute amount of dehydroascorbate plus dioxogulonate is really decreased. This implies that the catabolism of ascorbate in the liver is decreased. Whether there is also an increased synthesis cannot be deduced from the present work, but increased synthesis alone, without diminished catabolism, would if anything tend to lead to an increase in the amount of dehydroascorbate plus dioxogulonate. Since this work was completed Salomon & Stubbs (1961) have shown that the catabolism of ascorbic acid labelled with ^{14}C is decreased by administration of corticotrophin. Their work also shows decreased concentration and synthesis in the liver days or weeks after hypophysectomy.

It thus appears that, whereas corticotrophin specifically depletes adrenal-gland vitamin C, its

effects on the organism also include increased concentrations in blood, liver and other tissues, and involve quantities of vitamin C in excess of those lost by the adrenal. The increased concentration in the liver is probably associated with decreased catabolism of ascorbate: the results of Lahiri & Lloyd (1962) make it unlikely that loss of adrenal ascorbate involves oxidation (cf. Lloyd & Sinclair, 1953), and with the present results lend no support to the view that corticotrophin promotes the oxidation of ascorbate.

SUMMARY

1. The effects of various procedures on the concentrations of ascorbate and of dehydroascorbate plus dioxogulonate in blood and various tissues of anaesthetized rats have been investigated.

2. Laparotomy, haemorrhage and administration of corticotrophin increase the concentrations in blood.

3. The rise can be seen when the adrenals are excluded from the circulation, the highest concentrations being in blood from the inferior vena cava above the diaphragm; the femoral arteriovenous difference becomes increasingly negative.

4. The concentrations of ascorbate in several tissues (liver, spleen and kidney) are significantly increased in corticotrophin-treated animals, and the accompanying fall in the liver content of dehydroascorbate plus dioxogulonate is consistent with diminished ascorbate catabolism.

5. There is no support for the view that corticotrophin promotes the oxidation of ascorbate.

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Studies on the Biosynthesis of Porphyrin and Bacteriochlorophyll by *Rhodopseudomonas spheroides*

3. THE EFFECT OF THREONINE ON THE BIOSYNTHESIS OF HOMOSERINE AND METHIONINE*

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Threonine inhibits the biosynthesis of bacteriochlorophyll by illuminated suspensions of *Rhodopseudomonas spheroides* and greatly increases the excretion of coproporphyrin; ethionine is the only other compound tested that has a similar effect, and the biosynthesis of bacteriochlorophyll in the presence of threonine, or of ethionine, can be restored by the addition of homocysteine or methionine (Gibson, Neuberger & Tait, 1962*b*). Evidence was also presented that methionine donates its methyl group to form the methyl ester group of bacteriochlorophyll, and Tait & Gibson (1961) showed that chromatophore preparations catalyse the formation of magnesium protoporphyrin monomethyl ester from magnesium protoporphyrin and *S*-adenosylmethionine. The most likely explanation for the effect of ethionine was that its *S*-adenosyl derivative inhibits the

* Part 2: Gibson, Neuberger & Tait (1962*b*).