SUMMARY

1. The effects of disulphides on glucose utilization in various rat tissues *in vitro* as well as on crude calf-brain hexokinase have been studied.

2. High concentrations of disulphides inhibit the utilization of glucose in diaphragm, brain, testicle and kidney, whereas the production of lactic acid from endogenous substrates is not inhibited.

3. Crude calf-brain hexokinase is inhibited by disulphides to the same extent as is the utilization of glucose in brain-tissue slices. Both inhibitions can be partially reversed by thiols.

4. The inhibition is tentatively ascribed to mixed disulphide formation on essential thiol groups of hexokinase.

5. Glucose and to a limited extent the adenosine triphosphate-Mg²⁺ ion complex protect crude calf-brain hexokinase against the inhibition by disulphides.

6. The apparent specificity of the enzyme inhibitory action of disulphides of low oxidation-reduction potentials, as well as the protective mechanism of the cells against this inhibition, are discussed.

This work has been supported by U.S. Public Health Service (grant no. A3891). R.N. is a Fellow of the Norwegian Cancer Society.

REFERENCES

- Barker, S. B. & Summerson, W. H. (1941). *J. biol. Chem.* **138**, 535.
- Barron, E. S. G. (1951). *Advanc. Enzymol.* **11**, 201.
- Bhattacharya, S. K. (1959). *Nature, Lond.*, **184**, 1638.
- Boyer, P. D. (1959). In *The Enzymes*, 2nd ed., vol. 1, p. 511. Ed. by Boyer, P. D., Lardy, H. & Myrback, K. New York: Academic Press Inc.
- Butterfield, W. J. H. (1960). In *The Mechanism of Action of Insulin*, p. 247. Ed. by Young, F. G., Broom, W. A. & Wolff, F. W. Oxford: Blackwell Scientific Publications.
- Caramia, F. (1961). *Sperimentale*, **111**, 97.
- Ciasca, G. (1959). *Minerva ginec.* **11**, 185.
- Crane, R. K. & Sols, A. (1955). In *Methods in Enzymology*, vol. 1, p. 277. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Dickens, F. & Williamson, D. H. (1956). *Biochem. J.* **64**, 567.
- Dixon, M. & Needham, D. M. (1946). *Nature, Lond.*, **158**, 432.
- Eldjarn, L. (1954). *Scand. J. clin. Lab. Invest.* **6**, suppl. 13, p. 18.
- Eldjarn, L. & Bremer, J. (1962). *Biochem. J.* **84**, 286.
- Eldjarn, L., Bremer, J. & Børresen, H. C. (1962). *Biochem. J.* **82**, 192.
- Eldjarn, L. & Pihl, A. (1956). *J. biol. Chem.* **223**, 341.
- Eldjarn, L. & Pihl, A. (1957). *J. biol. Chem.* **225**, 499.
- Elliott, K. A. C. (1955). In *Methods in Enzymology*, vol. 1, p. 3. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
- Gilman, A., Philips, F. S., Koelle, E. S., Allen, R. P. & St John, E. (1946). *Amer. J. Physiol.* **147**, 115.
- Griffiths, M. (1949). *Arch. Biochem.* **20**, 451.
- Hultman, E. (1959). *Nature, Lond.*, **183**, 108.
- Mellwain, H. (1959a). *Biochemistry and the Central Nervous System*, 2nd ed., p. 32. London: J. and A. Churchill Ltd.
- Mellwain, H. (1959b). *Biochem. J.* **71**, 281.
- Pihl, A. & Eldjarn, L. (1958). *Proc. 4th int. Congr. Biochem., Vienna*, vol. 13, p. 43. London: Pergamon Press Ltd.
- Pihl, A. & Lange, R. (1962). *J. biol. Chem.* **237**, 1356.
- Rall, T. W. & Lehninger, A. L. (1952). *J. biol. Chem.* **194**, 119.
- Scheuch, D. & Rapoport, S. (1962). *Acta biol. med. german.* **8**, 31.
- Sols, A. & Crane, R. K. (1954). *J. biol. Chem.* **206**, 925.
- Ström, G. (1949). *Acta physiol. scand.* **17**, 440.
- Strömme, J. H. (1963). *Biochem. Pharmacol.* **12**, 157.
- Villar-Palasi, C., Carballido, A., Sols, A. & Arteta, J. L. (1957). *Nature, Lond.*, **180**, 387.

Biochem. J. (1963) **87**, 532

Periodic Activation of Lysosomal Enzymes during Regeneration of the Liver

BY R. L. P. ADAMS

Department of Biochemistry, University of Oxford

(Received 14 November 1962)

It has been suggested that an increase in the acid deoxyribonuclease content of a tissue is responsible for, or is directly associated with, an increase in the rate of cell division (Stern, 1960; Brody & Thorell, 1957). It is visualized that this would take place by a stimulation of DNA synthesis after the increase in concentration of precursors.

Acid deoxyribonuclease occurs in the lysosomes together with several other hydrolases, which also have an acid pH optimum (de Duve, 1959). These enzymes exhibit latency, i.e. when rat liver is gently homogenized in 0.25M-sucrose the enzymes exhibit only a fraction of the activity shown in the presence of 0.1% Triton X-100. Total deoxyribo-

nuclease activity is reported to be high in dividing tissues such as regenerating liver (Brody, 1958; Goutier & Goutier-Piotte, 1961; Goutier-Piotte & Goutier, 1962), but the activity in the absence of Triton X-100 (free activity) does not rise proportionally. It is the percentage free activity that reflects the permeability of the lysosomes.

Several other treatments are known to affect the pattern of lysosomal permeability (de Duve, Wattiaux & Wibo, 1962; Bitensky & Gahan, 1962); both arylsulphatase (Ugazio, 1960) and β -glucuronidase (Levy, Kerr & Campbell, 1948) undergo changes in distribution corresponding to changes in the amount of cell division in the tissue.

It was of interest to know whether periodic changes in permeability of the lysosomes were a characteristic of a synchronously dividing cell population, and also whether the concentration and distribution of different lysosomal enzymes changed independently under such conditions. This paper is a study of the activities of several lysosomal enzymes in rat liver after partial hepatectomy.

METHODS

White Wistar rats were used throughout. Partial hepatectomies were performed by the method of Higgins & Anderson (1931). The rats were killed by cervical fracture and the livers were homogenized in ice-cold buffered 0.25 M-sucrose, with two strokes of a glass-and-Teflon homogenizer of the Potter-Elvehjem type having a clearance across the diameter of 300 μ and a speed of 1000 rev./min. The homogenate was filtered through muslin before use. Total activity is that measured in the presence of 0.1% of Triton X-100 (supplied by Charles Lennig and Co. Ltd., 26-28 Bedford Row, London, W.C. 1).

The enzymic activity of the homogenate is based on its DNA phosphorus content, which was estimated by Burton's (1956) method.

Acid deoxyribonuclease. This was measured by the method described by de Duve, Pressman, Gianetto, Wattiaux & Appelmans (1955). The substrate was a solution containing 6 mg. of DNA (British Drug Houses Ltd.)/

ml. of 0.25 M-sucrose buffered to pH 5.0 with 0.2 M-acetate buffer, and containing 0.02 M-MgCl₂. This was dialysed for 24 hr. against a similar buffered sucrose solution to remove contaminants of low molecular weight. A portion (0.25 ml.) of substrate was incubated for 0 or 15 min. at 37° with 0.25 ml. of 20% (w/v) homogenate. The reaction was stopped by addition of an equal volume of ice-cold 10% (v/v) HClO₄. The tubes were spun after 10 min. and the extinction of the supernatant was measured at 260 m μ . The enzyme activity is expressed as μ m-moles of thymidine liberated during incubation for 15 min.

Acid phosphatase. This was measured by the method of Goodlad & Mills (1957). A portion (0.25 ml.) of 0.05 M-sodium β -glycerophosphate in 0.25 M-sucrose buffered to pH 5.0 with 0.1 M-acetate buffer was incubated at 37° for 0 or 10 min. with 0.25 ml. of 20% (w/v) homogenate and the reaction stopped by addition of 1.0 ml. of ice-cold 10% (w/v) trichloroacetic acid. Inorganic phosphate was measured in the supernatant by the method of Chen, Toribara & Warner (1956).

Arylsulphatase B. This was measured by the method of Roy (1953), with nitrocatechol sulphate prepared by the method of Roy (1958). In the assay described 100 μ g. of nitrocatechol gives extinction 0.7 at 520 m μ , and this was used for reference.

β -Glucuronidase. This was measured by the method of Gianetto & de Duve (1955). A portion (0.25 ml.) of 2.5 mm-phenolphthalein glucuronide (L. Light and Co. Ltd.) in 0.25 M-sucrose buffered to pH 5.2 with 75 mM-acetate buffer, was incubated at 37° with 0.25 ml. of 10% (w/v) homogenate. The reaction was stopped after 2 or 7 min. by addition of 3 ml. of cold 0.133 M-glycine buffer, pH 10.7. The extinction produced by the phenolphthalein liberated into the supernatant was measured at 550 m μ . Precipitation was improved by addition of a little CHCl₃. The reaction was linear over the period measured.

RESULTS

Changes in the total and free enzyme activities with regeneration are shown in Tables 1-3. Fig. 1 shows the changes in percentage free activity of the four enzymes. Gianetto & de Duve (1955) report that 0.25 M-sucrose has an inhibitory effect on the action of β -glucuronidase but, as in their work, no

Table 1. *Change in total enzyme activity with time of regeneration in rat liver*

Acid-deoxyribonuclease activity is expressed as m-moles of thymidine liberated/g. of DNA phosphorus/15 min. incubation. Arylsulphatase activity is expressed as g. of nitrocatechol liberated/g. of DNA phosphorus/15 min. incubation. β -Glucuronidase activity is expressed as g. of phenolphthalein liberated/g. of DNA phosphorus/5 min. incubation. Total activity is obtained by incubation in the presence of 0.1% (v/v) Triton X-100. Results are expressed $\pm$ s.d., and figures in parentheses are the numbers of determinations and the significance of difference between the mean value of the group and the mean value of the control group. These *P* values were obtained by Student's *t* test for small samples. For details of the assays see the Methods section.

Time after partial hepatectomy (hr.)	...	Control	14	20	23	26	29
Acid deoxyribonuclease	10.5±1.7 (14)	13.8±0.7 (4) (0.01-0.05)	15.2±0.7 (6) (0.01-0.05)	11.7±2.4 (8) (> 0.5)	11.6±2.5 (12) (> 0.5)	11.7±3.3 (10) (> 0.5)	
Arylsulphatase	3.7±0.1 (16)	4.2±1.1 (4) (0.01-0.05)	3.3±0.3 (4) (0.1)	2.5±0.2 (6) (< 0.001)	2.6±0.2 (6) (< 0.001)	2.4±0.2 (4) (< 0.001)	
β-Glucuronidase	3.4±0.6 (10)	5.9±0.9 (12) (0.001)	3.2±0.2 (6) (0.1-0.5)	4.1±2.7 (12) (> 0.5)	4.3±2.2 (6) (> 0.5)	3.7±1.4 (12) (> 0.5)	

account has been taken of this in the present paper. Wattiaux & de Duve (1956) remark that Triton X-100 can interfere with the estimation of inorganic phosphate, but no such difficulty was encountered here.

There is a 50% rise in the total activity of β -glucuronidase 14 hr. after partial hepatectomy and significant changes occur in the total activities of deoxyribonuclease and arylsulphatase at this time. Acid-deoxyribonuclease and acid-phosphatase activities undergo 50 and 200% increases respectively, to reach maxima at 20 hr. Arylsulphatase and β -glucuronidase activities are normal at this time, but both total and free arylsulphatase activities are decreased from 20 hr.

Free acid-phosphatase activity continues to rise until 26 hr. after hepatectomy, when it represents

50% of the total activity. By 29 hr. there is a marked drop in both the total and the free activity. To see whether these changes were in any way related to the mitotic cycle in regenerating liver the studies with acid phosphatase were extended to the time of the second mitotic peak (50–56 hr.). It can be seen (Table 3) that a similar series of changes occurs. The total activity rises from 29 hr. after hepatectomy until 51 hr. and then it begins to fall. The free activity, however, continues to rise until 54 hr. after the operation. Although increases in the percentage free activity occur at 26 hr. and 54 hr. they are not statistically significant.

There is only a slight change in distribution of arylsulphatase activity after partial hepatectomy but the changes that do occur parallel those of β -

Table 2. *Changes in free enzyme activity with time of regeneration in rat liver*

Free activity is that measured in the homogenate in 0.25M-sucrose in the absence of Triton X-100. Units of activity are as described in Table 1, and the details of the assays are described in the Methods section.

Time after partial hepatectomy (hr.) ...	Control	14	20	23	26	29
Acid deoxyribonuclease	7.1 $\pm$ 2.1 (14)	8.8 $\pm$ 0.7 (4) (0.5)	8.6 $\pm$ 1.2 (6) ($>$ 0.5)	7.5 $\pm$ 3.2 (8) ($>$ 0.5)	7.5 $\pm$ 1.7 (12) ($>$ 0.5)	9.6 $\pm$ 3.2 (10) (0.1–0.5)
Arylsulphatase	2.4 $\pm$ 0.1 (16)	3.0 $\pm$ 0.1 (4) ($<$ 0.001)	2.0 $\pm$ 0.1 (4) ($<$ 0.001)	1.5 $\pm$ 0.1 (6) ($<$ 0.001)	1.7 $\pm$ 0.1 (6) ($<$ 0.001)	1.6 $\pm$ 0.1 (4) ($<$ 0.001)
β -Glucuronidase	1.6 $\pm$ 0.3 (10)	3.4 $\pm$ 0.4 (12) ($<$ 0.001)	1.7 $\pm$ 0.5 (6) ($>$ 0.5)	2.0 $\pm$ 1.3 (12) ($>$ 0.5)	2.6 $\pm$ 1.2 (6) (0.01–0.05)	2.2 $\pm$ 0.9 (12) (0.01–0.05)

Table 3. *Changes in total, free and percentage free activity of acid phosphatase in regenerating rat liver*

Percentage free activity is the free activity as a percentage of the total activity. Free activity is that measured in the homogenate in 0.25M-sucrose in the absence of Triton X-100 and total activity that measured in the presence of 0.1% Triton X-100. Activity is expressed as g. of inorganic phosphorus liberated/g. of DNA phosphorus/10 min. incubation. Details of the assay are described in the Methods section. In parentheses are values of *P* obtained by a comparison by Student's *t* test with the controls.

Time after partial hepatectomy (hr.)	Total	Free	Percentage free activity	No. of determin- ations
Control	5.6 $\pm$ 2.1	2.3 $\pm$ 0.2	41 $\pm$ 3.5	12
14	6.0 $\pm$ 2.3 ($>$ 0.5)	2.0 $\pm$ 1.1 ($>$ 0.5)	33 $\pm$ 6.5 ($>$ 0.5)	12
17	12.0 $\pm$ 2.2 (0.05–0.1)	4.3 $\pm$ 2.6 (0.1–0.5)	36 $\pm$ 7.5 ($>$ 0.5)	12
20	14.7 $\pm$ 5.8 (0.05–0.1)	5.2 $\pm$ 2.0 (0.001–0.01)	35 $\pm$ 6.5 ($>$ 0.5)	10
23	10.5 $\pm$ 1.0 (0.01–0.05)	3.4 $\pm$ 1.0 (0.01–0.05)	32 $\pm$ 5.3 ($>$ 0.5)	6
26	13.6 $\pm$ 1.7 (0.1–0.5)	6.9 $\pm$ 2.8 (0.01–0.05)	51 $\pm$ 12.4 (0.1–0.5)	18
29	5.9 $\pm$ 1.2 ($>$ 0.5)	3.0 $\pm$ 1.0 ($>$ 0.5)	51 $\pm$ 4.1 (0.1–0.5)	6
36	6.5 $\pm$ 2.9 ($>$ 0.5)	2.2 $\pm$ 1.0 ($>$ 0.5)	34 $\pm$ 6.1 ($>$ 0.5)	6
51	16.6 $\pm$ 1.3 (0.01–0.05)	4.7 $\pm$ 2.2 (0.05)	28 $\pm$ 12.5 ($>$ 0.5)	12
54	13.3 $\pm$ 1.4 (0.001–0.01)	7.1 $\pm$ 1.6 (0.001)	54 $\pm$ 11.5 (0.1–0.5)	6
57	8.0 $\pm$ 2.2 (0.1–0.5)	3.4 $\pm$ 0.2 ($>$ 0.5)	43 $\pm$ 4.1 ($>$ 0.5)	12

Fig. 1. Changes after partial hepatectomy in the percentage free activity of four lysosomal enzymes. ○, Acid deoxyribonuclease; △, acid phosphatase; ●, arylsulphatase; ▲, β-glucuronidase. For details of the assays see Methods section. Percentage free activity is the activity measured in the absence of Triton X-100 expressed as a percentage of the activity measured in the presence of 0.1% of Triton X-100.

glucuronidase. After a rise at 14 hr. there is a slight decrease in the percentage free activity, which reaches a minimum at 20–24 hr. and then increases at 26–29 hr. after hepatectomy.

DISCUSSION

The exact time-courses of DNA synthesis and mitosis in regenerating rat liver vary with conditions and particularly with the age of the rats and the time of day at which they are hepatectomized. Using the same stock of rats as used in the experiments reported here, Jaffe, Lajtha, Lascelles, Ord & Stocken (1959) injected 50 μc of inorganic [³²P]phosphate/100 g. body wt. 30 min. before death and measured the specific activity of liver DNA and the mitotic index of the liver at various times after partial hepatectomy. The former showed a peak at 24 hr. and the latter at 28 hr. The values then decreased until 48 hr. Hecht & Potter (1956) showed that the specific activity of DNA rises at 24 hr. and after 48 hr. and that the total DNA content of the liver rises at about 25 hr. and 50 hr. after hepatectomy. In the present experiments rats of 150–200 g. were used and as far as possible operations were carried out between 9 and 11 a.m.

The fact that arylsulphatase shows negligible changes in distribution after partial hepatectomy is further evidence that it is not contained in the same particles as are the typical lysosomal enzymes. Franklin (1962) discusses several experiments which lead to similar conclusions.

The rise in free β-glucuronidase activity 14 hr. after hepatectomy is interesting in view of the possible role of this enzyme in releasing oestradiol from the glucuronide conjugated form in which it is transported in the blood. Oestradiol is known to have growth-promoting effects and de Duve *et al.* (1962) have shown it to stimulate the release of bound acid phosphatase from lysosomes. β-Glucuronidase is present in a spectrum of particles ranging in size from microsomes to mitochondria (Wattiaux, Baudhuin, Berleur & de Duve, 1956), and a rise in microsomal β-glucuronidase at 14 hr. could be reconciled with the increased metabolism of these particles at this time (Decken & Hultin, 1958). The release of oestradiol into the liver cell caused by this increased activity of β-glucuronidase could be the cause of the rise in free activity of the lysosomal enzymes that is seen 26–29 hr. after hepatectomy. This peak in lysosomal permeability coincides with mitosis, as does the peak in percentage free acid-phosphatase activity at 54–57 hr. after hepatectomy.

The increases in total deoxyribonuclease and acid-phosphatase activities with maxima at 14–20 and 17–26 hr. respectively agree with the results of Goutier-Pirotte & Goutier (1962). Brody (1958) obtained the peaks for total deoxyribonuclease activity at 24 hr. and at 41 hr. Thus the increases in total deoxyribonuclease activity precede DNA synthesis by several hours and the enzyme may be concerned in some priming reaction such as suggested by Keir & Aird (1962) and Keir (1962).

It is suggested that the changes occurring at the time of cell division are caused by a cessation in the synthesis of new lysosomes, thus resulting in a preponderance of old, permeable particles. This is consistent with the fall in total activities that is observed before the fall in free activities.

SUMMARY

1. Total and free activities of several lysosomal enzymes have been measured after partial hepatectomy.

2. Total acid-phosphatase and acid-deoxyribonuclease activities rise to maxima 3–5 hr. before the time of maximal DNA synthesis.

3. The percentage free activities rise at the time of mitosis.

Thanks are due to Dr M. G. Ord and Dr L. A. Stocken for helpful discussion and to Dr T. J. Franklin for the gift of the nitrocatechol sulphate. The author is grateful to the

Medical Research Council and to the Christopher Welch Trustees for grants in support of this work. Financial support was also provided by the Department of Scientific and Industrial Research, the Rockefeller Foundation and the National Institutes of Health, Grant no. A-3369.

REFERENCES

- Bitensky, L. & Gahan, P. B. (1962). *Biochem. J.* **84**, 13 p.
 Brody, S. (1958). *Nature, Lond.*, **182**, 1386.
 Brody, S. & Thorell, B. (1957). *Biochim. biophys. Acta*, **25**, 579.
 Burton, K. (1956). *Biochem. J.* **62**, 315.
 Chen, P. S., Toribara, J. Y. & Warner, H. (1956). *Analyt. Chem.* **28**, 1756.
 Decken, A. von der & Hultin, T. (1958). *Exp. Cell Res.* **14**, 88.
 de Duve, C. (1959). In *Subcellular Particles*, p. 128. Ed. by Hayashi, T. New York: Ronald Press Co.
 de Duve, C., Pressman, B. C., Gianetto, R., Wattiaux, R. & Appelmans, F. (1955). *Biochem. J.* **60**, 604.
 de Duve, C., Wattiaux, R. & Wibo, M. (1962). *Biochem. Pharmacol.* **9**, 97.
 Franklin, T. J. (1962). *Biochem. J.* **82**, 118.
 Gianetto, R. & de Duve, C. (1955). *Biochem. J.* **59**, 433.
 Goodlad, G. A. J. & Mills, G. J. (1957). *Biochem. J.* **66**, 342.
 Goutier, R. & Goutier-Pirotte, M. (1961). *Nature, Lond.*, **192**, 371.
 Goutier-Pirotte, M. & Goutier, R. (1962). *Radiat. Res.* **16**, 728.
 Hecht, L. I. & Potter, V. R. (1956). *Cancer Res.* **16**, 988.
 Higgins, G. M. & Anderson, R. M. (1931). *Arch. Path.* **12**, 186.
 Jaffe, J. J., Lajtha, L. G., Lascelles, J., Ord, M. G. & Stocken, L. A. (1959). *Int. J. Radiat. Biol.* **1**, 241.
 Keir, H. M. (1962). *Biochem. J.* **84**, 44 p.
 Keir, H. M. & Aird, G. L. (1962). *Biochem. J.* **84**, 44 p.
 Levy, G. A., Kerr, L. M. H. & Campbell, J. G. (1948). *Biochem. J.* **42**, 462.
 Roy, A. B. (1953). *Biochem. J.* **53**, 12.
 Roy, A. B. (1958). *Biochem. J.* **68**, 519.
 Stern, H. (1960). *Ann. N.Y. Acad. Sci.* **90**, 440.
 Ugazio, G. (1960). *G. Biochim.* **9**, 98.
 Wattiaux, R., Baudhuin, P., Berleux, A. & de Duve, C. (1956). *Biochem. J.* **63**, 608.
 Wattiaux, R. & de Duve, C. (1956). *Biochem. J.* **63**, 606.

Biochem. J. (1963) **87**, 536

Effect of Liver Necrosis and Thyrotoxicosis on the Synthesis of L-Ascorbic Acid by Rat-Liver Microsomes

BY N. C. GHOSH AND N. C. KAR

Department of Applied Chemistry, University Colleges of Science and Technology, Calcutta 9, India

(Received 8 October 1962)

The administration of carbon tetrachloride can neither increase the liver-tissue reserve of ascorbic acid nor bring about an enhanced urinary excretion of ascorbic acid in rats (N. C. Ghosh & N. C. Kar, unpublished work). Lindan & Work (1953) observed that the ascorbic acid content of the livers of rats given a necrogenic diet was significantly lower than that of livers of rats given a 25% casein diet. They found that the preneurotic liver had a slightly diminished ascorbic acid content, and that when necrosis developed ascorbic acid could not be detected in the liver.

Various investigators have shown that the synthesis of ascorbic acid as evidenced from the urinary excretion is correlated with the activity of the endocrine glands. Dumm & Ralli (1949) observed that the amount of ascorbic acid excreted by rats after adrenalectomy falls to a very low value. Sure & Theis (1938) reported that, when thyroxine at a toxic dose is fed to rats, the ascorbic acid content of the adrenals, thymus and kidney is greatly decreased. Burns, Evans & Trousof (1957) demonstrated that barbital and Chloretone have little or no effect on ascorbic acid excretion in

hypophysectomized rats, suggesting a possible hormonal control of ascorbic acid synthesis in response to drugs.

The enzyme system catalysing the conversion of D-glucuronolactone and L-gulonolactone into L-ascorbic acid in animals is entirely located in the microsomal fractions of the rat- and goat-liver homogenates (Burns, Peyser & Moltz, 1956; Chatterjee, Ghosh, Ghosh & Guha, 1957*a, b*, 1958). Chatterjee, Kar, Ghosh & Guha (1961) also reported that the enzymic activity of liver microsomes for the conversion of L-gulonolactone into L-ascorbic acid is significantly decreased in vitamin E deficiency. The activity could be restored by the addition of α -tocopherol or vitamin K₁. A similar reactivation by α -tocopherol has also been obtained by Carpenter, Kitabchi, McKay & Caputto (1959), who observed an increased peroxide formation in the microsomes from vitamin E-deficient rats. They concluded that α -tocopherol functions by inhibiting the peroxide formation. Chatterjee *et al.* (1961) further showed that compounds other than antioxidants equally stimulate the synthesis of L-ascorbic acid by the microsomes from vitamin