- Arroyave, G., Wilson, D., Mendez, J., Behar, M. & Scrimshaw, N. S. (1961b). *Amer. J. clin. Nutr.* **9**, 180.
- Bagehi, K., Halder, K. & Chowdhury, S. R. (1959). *J. Indian med. Ass.* **33**, 401.
- Cravioto, J., De La Pena, C. L. & Burgos, C. (1959). *Metabolism*, **8**, 722.
- Davies, J. N. P. (1948). *Lancet*, **i**, 317.
- Dzialoszynski, L. M., Mystkowski, E. M. & Stewart, C. P. (1945). *Biochem. J.* **39**, 63.
- Esh, G. C. & Bhattacharya, R. K. (1960). *Proc. Symposium on Proteins, Mysore*, p. 107. Delhi: Council of Scientific and Industrial Research (India).
- Esh, G. C., Bhattacharya, R. K. & Basu, U. P. (1961). *Sci. & Cult.* **27**, 87.
- Friend, C. J., Heard, C. R. C., Platt, B. S., Stewart, R. J. C. & Turner, M. R. (1961). *Brit. J. Nutr.* **15**, 231.
- Ganguly, J. & Krinsky, N. I. (1953). *Biochem. J.* **54**, 177.
- Ganguly, J., Krinsky, N. I., Mehl, J. W. & Deuel, H. J., jun. (1952). *Arch. Biochem. Biophys.* **38**, 275.
- Ganguly, J., Krishnamurthy, S. & Mahadevan, S. (1959). *Biochem. J.* **71**, 756.
- Hiller, A. (1953). *Practical Clinical Chemistry*, p. 135. Springfield, Ill.: Charles C. Thomas.
- Jagannathan, S. N. & Patwardhan, V. N. (1960). *Indian J. med. Res.* **48**, 785.
- Krinsky, N. I., Cornwell, D. G. & Oncley, J. L. (1958). *Arch. Biochem. Biophys.* **73**, 233.
- Lyman, R. L. & Wilcox, S. S. (1960). *J. Nutr.* **72**, 265.
- McPhedran, N. T. & Lucas, C. C. (1960). *Surg. Forum*, **11**, 369.
- Mahadevan, S., Murthy, S. K. & Ganguly, J. (1962). *Biochem. J.* **85**, 326.
- Mahadevan, S., Murthy, S. K., Krishnamurthy, S. & Ganguly, J. (1961). *Biochem. J.* **79**, 416.
- Mahadevan, S., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **88**, 534.
- Malathi, P., Subba Rao, K., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **87**, 305.
- Muramatsu, K. & Ashida, K. (1962). *J. Nutr.* **76**, 143.
- Olson, R. E., Jablonski, J. R. & Taylor, E. (1958). *Amer. J. clin. Nutr.* **6**, 111.
- Price, R. W. (1962). *Aerospace Med.* **33**, 42.
- Ramakrishnan, S., Srinivasan, V. & Nedungadi, T. M. B. (1961). *J. Nutr.* **75**, 443.
- Reichigl, M., jun., Berger, S., Loosli, J. K. & Williams, H. H. (1962). *J. Nutr.* **76**, 435.
- Robinson, H. W. & Hogden, C. G. (1940). *J. biol. Chem.* **135**, 727.
- Steenbock, H. & Nelson, E. M. (1923). *J. biol. Chem.* **56**, 355.
- Tejada, C. (1956). *Rev. Col. méd., Guatemala*, **7**, 235.
- Thompson, M. D. & Trowell, H. C. (1952). *Lancet*, **i**, 1031.
- Waterlow, J. C. (1959). *Fed. Proc.* **18**, 1143.

Biochem. J. (1964) **90**, 104

Studies on Metabolism of Vitamin A

6. THE EFFECT OF VITAMIN A DEFICIENCY ON THE ACTIVATION OF SULPHATE AND ITS TRANSFER TO *p*-NITROPHENOL IN RAT LIVER

By K. SUBBA RAO AND J. GANGULY

Department of Biochemistry, Indian Institute of Science, Bangalore 12, India

(Received 19 April 1963)

It is generally believed that sulphate metabolism is dependent on the vitamin A nutritional status of the animal (Fell, Mellanby & Pele, 1956; Dziewiatkowski, 1954; Balakhovskii & Kuznetsova, 1958). Wolf & Varandani (1960) have reported that vitamin A deficiency leads to a decreased incorporation of [³⁵S]sulphate into the mucopolysaccharides of colon homogenates of rats. This was claimed to be due to a lowered synthesis of 'active sulphate' (adenosine 3'-phosphate 5'-sulphatophosphate) and not to any loss of the activity of the enzyme transferring the sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to mucopolysaccharides (Varandani, Wolf & Johnson, 1960). It has been shown that the sulphurylation of *p*-nitrophenol is markedly decreased in the tissues of vitamin A-deficient rats (Subba Rao, Seshadri Sastry & Ganguly, 1963). Sulphurylation of phenols is a two-step reaction, the first being the activation of sulphate

by way of the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate, and the second the transfer of sulphate from the adenosine 3'-phosphate 5'-sulphatophosphate to phenols catalysed by phenol sulphokinase (De Meio, Wizerkaniuk & Schreiberman 1955; Spencer, 1960; Gregory & Robbins, 1960). In the present paper it is demonstrated that the rates of both the steps involved in the sulphurylation of phenols are decreased in the liver of the vitamin A-deficient rats, and it is further shown that the lost activity can be restored by the addition of vitamin A to the reaction mixture *in vitro*, the restoration being dependent on the form of vitamin A used.

MATERIALS AND METHODS

Animals. Male rats of this Institute strain, made vitamin A-deficient according to the procedure described by Malathi, Subba Rao, Seshadri Sastry & Ganguly (1963).

were used when they had lost 2-3 g. after the weight-plateau stage.

Chemicals. The sources of the chemicals used were as described by Subba Rao *et al.* (1963). Chondroitin sulphate, prepared from bovine nasal septa, was obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.

Preparation and assay of enzymes forming adenosine 3'-phosphate 5'-sulphatophosphate and transferring sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to *p*-nitrophenol. Rats were killed by a blow on the head and the livers were immediately removed into beakers placed in crushed ice. Liver homogenates (10%), prepared in 0.15M-KCl with a Potter-Elvehjem homogenizer, were centrifuged for 1 hr. at 100 000 *g* in a Spinco model L ultracentrifuge and the particle-free supernatant was used as the source of both enzymes.

Assay of the enzyme forming adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate. For routine procedure, the reaction mixture contained 0.3 μ -mole of carrier-free Na₂³⁵SO₄ (6.0 $\times$ 10⁵ counts/min., as measured in the scaler used in this study), 10 μ moles of cysteine, 10 μ moles of ATP (sodium salt), 10 μ moles of MgCl₂, 80 μ moles of phosphate buffer, pH 6.5, and about 2 mg. of enzyme protein in a total volume of 1 ml. Incubation was for 1 hr. at 37°. The reaction was terminated by heating the tubes in a boiling-water bath for 1 min., and the tubes were then immediately transferred into crushed ice. The protein was centrifuged off and a sample of the supernatant was analysed for adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate by paper electrophoresis according to the method of Varandani *et al.* (1960). The electrophoresis was run with Whatman no. 3MM paper strips (2.5 cm. $\times$ 46 cm.) in 50 mM-citrate buffer, pH 5.6, at 300 v for 5½ hr. After the run, the strips were dried in air, cut into 1 cm. pieces and scanned for radioactivity. Under these conditions adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate moved 20 cm. and inorganic sulphate 28 cm., which is in agreement with the observations of Varandani *et al.* (1960). The area corresponding to adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate was twice eluted with cold water, after which no radioactivity could be detected in the eluted paper. The purity of the adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate was ascertained from its absorption maxima at 260 m μ , from the fact that it is almost completely hydrolysed by 0.1N-HCl at 37° in 30 min. (Robbins & Lipmann, 1957) and from its ability to donate enzymically the sulphate group to *p*-nitrophenol, when measured as described below, except that the enzyme protein was about 2.5 mg. Under these conditions about 80% of the [³⁵S]-sulphate of adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate was transferred to *p*-nitrophenol. The total radioactivity of adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate synthesized was measured by plating a sample of the eluted adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate solution on aluminium planchets, followed by counting.

Assay of the transfer enzyme. The adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate used in this system was obtained by the above procedure, where proportionately larger amounts of all the constituents of the reaction mixture were used and the final eluent was freeze-dried.

The reaction mixture contained 50 000 counts/min. of adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate, 50 μ moles of *p*-nitrophenol, 5 μ moles of cysteine, 0.1 ml. of 0.4M-EDTA, 80 μ moles of phosphate buffer, pH 7.1, and about 2 mg. of enzyme protein in a total volume of 1 ml.

The reaction was terminated as before after incubation for 1 hr. at 37°. Protein was centrifuged off and a sample of the supernatant was subjected to paper chromatography for the isolation of *p*-nitrophenyl [³⁵S]sulphate according to the method of Balasubrahmanyam & Bachhawat (1962). Radioactivity was measured with a Geiger-Müller tube (General Electric Co. Ltd.: type E.H.M. I/S, window weight 2.3 mg./cm.², operating voltage 1520v), attached to a Panax (Redhill, Surrey) scaler (type 100 C). The counts expressed throughout the present paper were as obtained with this instrument; all countings were carried out at infinite thinness.

Determination of protein. Protein was estimated by the biuret method (Gornall, Bardawill & David, 1949).

Optimum pH for assay of the enzymes. In a previous report (Subba Rao *et al.* 1963), where the pH-activity relationship of the total reaction involving the sulphurylation of *p*-nitrophenol was studied, maximum activity was obtained within the pH range 7.6-8.0 with a minor peak at pH 6.0. Fig. 1 shows that the optimum pH for the activation step is at pH 6.5 and that for the transfer reaction at pH 7.1.

Fig. 1. Synthesis of adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate by rat-liver supernatant, and the transfer of [³⁵S]sulphate to *p*-nitrophenol from adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate. The reaction mixtures and assay conditions were as described in the Materials and Methods section. (a) Effect of pH on the formation of adenosine 3'-phosphate 5'-[³⁵S]-sulphatophosphate (protein 1.3 mg.). (b) Effect of pH on the formation of *p*-nitrophenyl [³⁵S]sulphate (protein 1.2 mg.).

Maximum duration for assay of the enzyme forming adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate. In these experiments with the rat-liver supernatant the formation of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate was linear up to 1½ hr. of incubation, after which the adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate concentration steadily decreased (Fig. 2); this is in agreement with the results of Spencer (1960).

RESULTS

Effect of vitamin A deficiency on the synthesis of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate and on the transfer of [³⁵S]sulphate to p-nitrophenol. Table 1 shows that there was a 30–35 % decrease in the synthesis of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate and also a similar loss of the

Fig. 2. Synthesis of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate by rat-liver supernatant. The reaction mixture and assay conditions were as described in the Materials and Methods section (protein 2.0 mg.).

transferase activity in the vitamin A-deficient rats. The addition of vitamin A alcohol *in vitro* fully restored the lost activity of the first step (synthesis of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate), and the acid was fully effective in the second (transfer of sulphate). Further, vitamin A acid had some effect on the first reaction; and vitamin A alcohol, though not as effective as the acid, enabled partial recovery of activity in the second.

Effect of the addition of graded amounts of vitamin A alcohol or acid *in vitro*. Table 2 shows that, whereas vitamin A alcohol was able to reverse the effect of the deficiency on the first reaction even at 2.5 µg., 10 µg. of the acid was required for the maximum effect that could be obtained for the same reaction. However, under conditions where the maximum activation could be obtained with the acid, it was only partially as effective as the alcohol.

Table 2 also shows that, though the lost activity of the second reaction was fully restored by 5 µg. of vitamin A acid, 10 µg. of the alcohol was required for the maximum effect. Here also, at the concentration where the alcohol showed the maximum effect, it was only partly as effective as the acid. At 20 µg. or above both compounds had inhibitory effects on both the reactions. Further, the addition of vitamin A alcohol or acid *in vitro* to the enzyme systems prepared from normal rat liver had no significant effect on the two reactions (Table 1).

Specificity of vitamin A. Experiments with a number of fat-soluble substances showed that vitamin A alcohol was the only compound able to bring back the lost activity of the first reaction to the normal level, and that vitamin A acid was the

Table 1. Effect of vitamin A deficiency on the synthesis of adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate and on the transfer of [³⁵S]sulphate to p-nitrophenol from adenosine 3'-phosphate 5-[³⁵S]-sulphatophosphate in rat liver

The assay of enzymic activities was carried out as described in the Materials and Methods section. The given amounts of vitamin A alcohol or acid were added to the reaction mixture. All values are averages of six separate experiments, with one rat for each trial. The ranges are given in parentheses.

Status of rat	Additions	Adenosine 3'-phosphate 5-[³⁵ S]-sulphatophosphate formed (counts per min./mg. of protein/hr.)	p-Nitrophenyl [³⁵ S]sulphate formed (counts per min./mg. of protein/hr.)
Normal	None	33 050 (29 000–38 900)	16 250 (14 000–20 130)
	5 µg. of vitamin A alcohol in 0.01 ml. of 1 % (v/v) ethanol	31 150 (25 000–37 600)	15 680 (13 850–20 000)
	5 µg. of vitamin A acid in 0.01 ml. of 0.2M-phosphate buffer, pH 10.0	30 980 (25 000–38 600)	16 000 (14 100–19 500)
Deficient	None	22 740 (19 000–27 500)	10 185 (8 200–12 500)
	5 µg. of vitamin A alcohol in 0.01 ml. of 1 % (v/v) ethanol	32 150 (27 000–34 400)	13 480 (11 000–15 000)
	5 µg. of vitamin A acid in 0.01 ml. of 0.2M-phosphate buffer, pH 10.0	25 000 (22 220–30 000)	15 500 (13 000–17 500)
	0.01 ml. of 1 % (v/v) ethanol	23 620 (19 000–28 400)	10 900 (9 000–12 300)

only compound fully effective for the second reaction (Table 3). However, retinene was partly active on both reactions, which could be due to its conversion into the alcohol or the acid (Mahadevan, Murthy & Ganguly, 1962).

Effect of the oral administration of vitamin A acetate or acid to vitamin A-deficient rats. Vitamin A acetate and acid seem to have similar effects in most metabolic functions in rats, except vision and perhaps reproduction (Moore, 1962; Thompson,

Table 2. *Effect of the addition of graded amounts of vitamin A alcohol or acid in vitro on the synthesis of adenosine 3'-phosphate 5' [³⁵S]-sulphatophosphate and p-nitrophenyl [³⁵S]sulphate in the liver of vitamin A-deficient rats*

The assay of enzymes was carried out as described in the Materials and Methods section. Where stated, the given amounts of vitamin A alcohol or acid were added in 0.01 ml. of 1% (v/v) ethanol or 0.01 ml. of 0.2M-phosphate buffer, pH 10.0, respectively. Each value represents the average of two experiments carried out with two different rats. The ranges are given in parentheses. N.D., Not determined.

Status of rat	Additions		Adenosine 3'-phosphate 5' [³⁵ S]-sulphatophosphate formed (counts per min./mg. of protein/hr.)	p-Nitrophenyl [³⁵ S]sulphate formed (counts per min./mg. of protein/hr.)
	Vitamin A alcohol (μg.)	Vitamin A acid (μg.)		
Normal	0	0	34 150 (33 700-34 600)	16 650 (16 000-17 300)
Deficient	0	0	20 500 (20 200-20 800)	8 180 (7 900- 8 460)
	2.5	0	35 900 (35 600-36 200)	N.D.
	5.0	0	35 300 (35 000-35 600)	14 540 (14 200-14 880)
	10.0	0	34 900 (33 900-35 900)	14 800 (14 500-15 100)
	20.0	0	20 000 (19 500-20 500)	10 900 (10 000-11 800)
	50.0	0	19 500 (17 600-21 400)	10 000 (9 500-10 500)
	0	2.5	N.D.	10 000 (9 000-11 000)
	0	5.0	23 530 (22 900-24 160)	17 000 (16 800-17 200)
	0	10.0	25 706 (25 412-26 000)	14 540 (14 240-14 840)
	0	20.0	21 470 (20 700-22 240)	11 810 (11 000-12 620)
	0	50.0	19 230 (19 000-19 460)	11 810 (11 200-12 420)

Table 3. *Specificity of vitamin A: the effect of the addition of several fat-soluble compounds in vitro on the synthesis of adenosine 3'-phosphate 5' [³⁵S]-sulphatophosphate and p-nitrophenyl [³⁵S]sulphate in the liver of vitamin A-deficient rats*

Enzymic activities were assayed as described in the Materials and Methods section. All the substances were added in 0.01 ml. of 1% (v/v) ethanol. Each value is the result of a single experiment. N.D., Not determined.

Status of rat	Additions (5 μg.)	Adenosine 3'-phosphate 5' [³⁵ S]-sulphatophosphate formed (counts per min./mg. of protein/hr.)	p-Nitrophenyl [³⁵ S]sulphate formed (counts per min./mg. of protein/hr.)
Normal	None	32 600	15 000
Deficient	None	21 000	11 800
	Vitamin A alcohol	32 000	N.D.
	Vitamin A acid	N.D.	15 720
	β-Carotene	23 600	10 500
	Retinene	29 700	13 500
	Vitamin E	21 000	10 500
	Vitamin D	20 000	9 800
	Menadione	17 700	10 250
	Cholesterol	19 470	10 610
	Palmitic acid	19 230	9 650

Howell & Pitt, 1961; Dowling & Wald, 1960). Malathi *et al.* (1963) have shown that the acid is remarkably more active in promoting the growth of vitamin A-deficient rats. When the vitamin A-deficient rats were given three doses of 100 μ g. each of the acid or the acetate on alternate days and were killed 24 hr. after the last dose, the activities of both enzymes of the liver showed no significant differences between the two groups and their levels were comparable with those obtained from normal animals. Since in these experiments the animals received large doses of the acid for a few days, it is possible that the cumulative effect of its residual activity was sufficient to counteract the influence of the deficiency.

Transfer of [35 S]sulphate from adenosine 3'-phosphate 5' [35 S]-sulphatophosphate to chondroitin sulphate. In separate experiments the transfer of [35 S]sulphate from adenosine 3'-phosphate 5' [35 S]-sulphatophosphate to chondroitin sulphate was studied. The reaction mixture and the other conditions were similar to those used for the formation of *p*-nitrophenyl [35 S]sulphate, except that 50 μ g. of chondroitin sulphate replaced the *p*-nitrophenol. The separation of chondroitin [35 S]sulphate was carried out according to the method of Suzuki & Strominger (1960). However, no transfer of [35 S]-sulphate from adenosine 3'-phosphate 5' [35 S]-sulphatophosphate to chondroitin sulphate was observed. It is possible that rat liver is devoid of the activity of transferring the sulphate to chondroitin sulphate.

DISCUSSION

The results confirm previous observations (Subba Rao *et al.* 1963) that vitamin A deficiency leads to a defective sulphurylation of phenols, and further show that both steps of sulphurylation are markedly decreased in the liver of vitamin A-deficient rats.

According to Varandani *et al.* (1960) vitamin A deficiency causes a decrease in the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate and not in the transfer of sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to mucopolysaccharides of rat-colon homogenates. Our results agree with this in that we have demonstrated that vitamin A deficiency leads to a decreased synthesis of adenosine 3'-phosphate 5'-sulphatophosphate in rat liver. In addition, we have shown that the transfer of sulphate to phenols is also decreased. However, enzymes catalysing the transfer of sulphate from adenosine 3'-phosphate 5'-sulphatophosphate to phenols and to mucopolysaccharides have been reported to be different (Gregory & Robbins, 1960). That these enzymes are different is further substantiated by our present observation that, though the supernatant fraction of normal rat liver catalysed the transfer of [35 S]sulphate to

p-nitrophenol, it failed to do so when chondroitin sulphate was the acceptor.

While this work was in progress Pasternak, Humphries & Pirie (1963) claimed that vitamin A deficiency does not affect the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate in the cornea and colonic mucosa of rats and rabbits. However, closer examination of the paper of Pasternak *et al.* (1963) reveals that their animals might not have been totally depleted of vitamin A. Thus it was stated that weanling rats were maintained on vitamin A-free diet for about 4 weeks, when the corneal changes began, and that at this stage their liver vitamin A content was too low to determine. It is well recognized that rats can grow well even if they do not have any detectable vitamin A in their liver (Baumann, Riising & Steenbock, 1934; Davis & Moore, 1948), which suggests that the absence of chemically detectable vitamin A from liver is not the final proof of total depletion of tissue vitamin A. On the other hand, a more accepted criterion for the total depletion in rats appears to be the failure to grow for four successive days or loss of weight (United States Pharmacopeia, 1950), rather than the corneal changes. As for the rabbits, they still contained some vitamin A in their livers. Further, the authors admit that adenosine 3'-phosphate 5' [35 S]-sulphatophosphate and chondroitin sulphate moved together in their system, in which case it would be difficult to interpret their results owing to the absence of any data on the net synthesis of adenosine 3'-phosphate 5' [35 S]-sulphatophosphate. The present paper, on the other hand, gives a measure of the net synthesis of adenosine 3'-phosphate 5' [35 S]-sulphatophosphate, because no transfer of [35 S]sulphate to chondroitin sulphate from adenosine 3'-phosphate 5' [35 S]-sulphatophosphate could be observed in rat liver. It should be pointed out that in the present work we have strictly followed the United States Pharmacopeia (1950) procedure for obtaining the vitamin A-deficient rats (Malathi *et al.* 1963); nevertheless, our results were obtained with rat liver, whereas Pasternak *et al.* (1963) used the cornea and colonic mucosa of rats and rabbits.

In previous work (Subba Rao *et al.* 1963), where the total reaction of sulphurylation of *p*-nitrophenol was studied with inorganic sulphate and *p*-nitrophenol, both vitamin A alcohol and acid were equally effective in restoring the activity lost due to deficiency. In the present work, where the activation and transfer reactions were studied separately, vitamin A alcohol was fully active in restoring the first reaction and partially in the second; the acid acted vice versa. Therefore it is possible that, when the inorganic sulphate was the substrate and *p*-nitrophenol the acceptor, the full reactivation of one step and partial reactivation of the other were

enough to drive the reaction towards the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate, because owing to the presence of an excess of the ready acceptor the adenosine 3'-phosphate 5'-sulphatophosphate formed would constantly be used up.

That vitamin A is specific for these reactions is borne out by the fact that, of the many fat-soluble compounds tried, only the different forms of vitamin A were able to restore *in vitro* the loss of the activity caused by the deficiency. Different forms of vitamin A are currently being assigned different physiological functions, e.g. the aldehyde for the vision, and the acid for general growth (Wolf, 1962; Malathi *et al.* 1963). It is now shown that, in the sulphurylation of phenols by rat liver, vitamin A alcohol and acid take part in the activation and transfer steps respectively.

SUMMARY

1. Both the steps involved in the sulphurylation of phenols, namely activation and transfer of sulphate, were significantly decreased in the liver supernatant of vitamin A-deficient rats. The addition of vitamin A alcohol *in vitro* was more effective in restoring the lost activity of the first step, whereas the acid was more effective in the second.

2. Of the several fat-soluble compounds tried, only the different forms of vitamin A were, in various degrees, active in restoring the lost activity.

3. After the oral administration of three large doses of vitamin A acetate or acid to vitamin A-deficient rats, both the enzyme activities of the liver of the two groups were found to be normal.

K.S.R. is a C.S.I.R. Junior Research Fellow. We thank the Williams-Waterman Fund of the Research Corporation, New York, for a grant for special chemicals.

REFERENCES

- Balakhovskii, S. D. & Kuznetsova, I. V. (1958). *Dokl. Akad. Nauk S.S.S.R.*, **118**, 331.
 Balasubrahmanyam, A. S. & Bachhawat, B. K. (1962). *Biochim. biophys. Acta*, **59**, 389.
 Baumann, C. A., Riising, B. M. & Steenbock, H. (1934). *J. biol. Chem.* **107**, 705.
 Davis, A. W. & Moore, T. (1948). *Biochem. J.* **42**, lxiii.
 De Meio, R. H., Wizerkaniuk, M. & Schreiberman, I. (1955). *J. biol. Chem.* **213**, 439.
 Dowling, J. E. & Wald, G. (1960). *Proc. nat. Acad. Sci., Wash.*, **46**, 587.
 Dziewiatkowski, D. S. (1954). *J. exp. Med.* **100**, 11.
 Fell, H. B., Mellanby, E. & Pelc, S. R. (1956). *J. Physiol.* **134**, 179.
 Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). *J. biol. Chem.* **177**, 751.
 Gregory, J. D. & Robbins, P. W. (1960). *Annu. Rev. Biochem.* **29**, 347.
 Mahadevan, S., Murthy, S. K. & Ganguly, J. (1962). *Biochem. J.* **85**, 326.
 Malathi, P., Subba Rao, K., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **87**, 305.
 Moore, T. (1962). *Nutr. Diets*, **3**, 1.
 Pasternak, C. A., Humphries, S. K. & Pirie, A. (1963). *Biochem. J.* **86**, 382.
 Robbins, P. W. & Lipmann, F. (1957). *J. biol. Chem.* **229**, 837.
 Spencer, B. (1960). *Biochem. J.* **77**, 294.
 Subba Rao, K., Seshadri Sastry, P. & Ganguly, J. (1963). *Biochem. J.* **87**, 312.
 Suzuki, S. & Strominger, J. L. (1960). *J. biol. Chem.* **235**, 257.
 Thompson, J. N., Howell, J. McC. & Pitt, G. A. J. (1961). *Biochem. J.* **80**, 25.
 United States Pharmacopeia (1950). 14th revision.
 Varandani, P. T., Wolf, G. & Johnson, C. (1960). *Biochem. biophys. Res. Commun.* **3**, 97.
 Wolf, G. (1962). *Nutr. Rev.* **20**, 161.
 Wolf, G. & Varandani, P. T. (1960). *Biochim. biophys. Acta*, **43**, 501.

Biochem. J. (1964) **90**, 109

The Changes in some Hydrolytic Enzymes in Carbon Tetrachloride-Induced Fatty Livers

By G. UGAZIO, MARIA ARTIZZU, P. PANI AND M. U. DIANZANI
Institute of General Pathology of the University of Cagliari, Italy

(Received 2 May 1963)

It is known that mitochondria from fatty livers are severely damaged. They are swollen and show a high degree of uncoupling of oxidative phosphorylation, as well as of activation of adenosine triphosphatase. Their permeability is increased. This fact provokes a partial transfer of certain coenzymes or

cofactors from the particles into the surrounding fluid (for references see Artizzu & Dianzani, 1962). In fatty liver the amount of soluble hydrolytic enzymes ('lysosomal' enzymes) increases. In some, such as cathepsin and ribonuclease, the specific activity of the hydrolytic enzymes is also increased