sible diminution, it was of special interest to examine the intrinsic activities of the two sympathomimetics under our experimental conditions. At 25°C both amines relaxed the preparation completely, that means the intrinsic activity amounted to 1.0. Troughout the experimental procedure, no change of the intrinsic activity for Th 1165a occured (Table). The intrinsic activity for IPN, on the other hand, decreased to 0.55 at 42°C and was only partly restored to 0.71 after recooling.

Discussion. Our results showed that the shift to the right of the dose-response curves for IPN and Th 1165a caused by an elevation of temperature from 25° to 42°C could not be completely reversed, i.e. an irreversible shift of the dose-response curves for the β-sympathomimetics remained after recooling to 25°C. This could be due to a denaturation of the contractile apparatus or to an actual conformational change of the β-receptors on rabbit ileum. The denaturation can be excluded since papaverine showed neither a diminution of affinity nor any alteration of the intrinsic activity at high temperature on rabbit ileum (unpublished results). We would like to suggest, therefore, that β-receptor sites undergo irreversible conformational changes. That accounts not only for adrenergic β-receptors but also for receptors mediating the responses for angiotensin, vasopressin[10] and serotonin[10,11] since on rat fundus strip, recooling from 47° to 37.5°C showed an irreversible shift of their dose-response curves to the right and also a decrease of the intrinsic activities. On the other hand, heating is without any influence on the dose-response curves for acetylcholine, KCl and bradykinin[10]. These results are in favour of the existence of heat-labile and -stable receptors on smooth muscle of the digestive tract.

According to our results, the irreversible part of the diminution of the sympathomimetic affinity is nearly equal for both amines. This implies that the population of the β-receptors which undergo an alteration of their active sites by our procedure is of the same order of magnitude.

On the other hand, the extent of the reversible diminution of affinity showed great differences for both amines. This part is greater for Th 1165a than for isoprenaline. That was not surprising since the decrease of affinity for Th 1165a[12] evoked by high temperatures was much more pronounced than for IPN. These results are in accordance with the observation that preincubation with the metabolic inhibitor, iodoacetic acid, increased the affinity of Th 1165a to a higher degree than for isoprenaline[7].

From the present study the conclusion can be drawn that in rabbit ileum the decrease of affinity induced by alteration of temperature is not only dependent upon an increase of the metabolic rate, as for instance in the guinea-pig atrium[3], but in addition by an irreversible impairment of the β-receptor sites.

The alteration of the intrinsic activity observed for isoprenaline cannot be explained so far and needs further elucidation by experiments.

Zusammenfassung. Am Kaninchen-Ileum besteht die durch Temperaturerhöhung bedingte Rechtsverschiebung der Dosis-Wirkungs-Kurven von Isoprenalin und Th 1165a aus einem reversiblen und einem irreversiblen Anteil.

D. REINHARDT, J. WAGNER and H. J. SCHÜMANN[13]

Pharmakologisches Institut der Universität Essen (Gesamthochschule) Hufelandstr. 55, D–43 Essen (Germany), 31 January 1973.

[10] J. H. FLEISCH and S. EHRENPREIS, J. Pharmac. exp. Ther. *162*, 21 (1968).
[11] A. VACCARI, Pharmacology *5*, 321 (1971).
[12] Our thanks are due to Dr. A. ENGELHARDT, C. H. Boehringer Ingelheim, for generous gift of the drugs.
[13] The authors wish to thank Mrs. GABRIELE GRAF and Miss BETTINA SCHEEPERS for skilful technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft.

Ascorbic Acid: Effect of High Doses on Brain and Heart Catecholamine Levels in Guinea-Pigs and Rats

The growing interest in megavitamin therapy for mental illness[1–4] has prompted us to study the effect of relatively high doses of ascorbic acid on catecholamine levels in the heart and brain of the guinea-pig, a species which like man, cannot synthesize its own ascorbic acid. THOA et al.[5] have shown that heart norepinephrine is decreased in the scorbutic guinea-pig while more recently LOKOSHKO and LESNYKH[6] have reported that in guinea-pigs with hypovitaminosis C, heart epinephrine is no different from normal healthy animals. In the present communication we report the effect of a relatively high dietary intake of vitamin C on heart and brain levels of catecholamines in the guinea-pig. By way of comparison, we also report the effect of a high dietary intake of ascorbic acid in the rat, a species which is capable of synthesizing its own ascorbic acid.

Materials and methods. Ascorbic acid-free powdered Reid-Briggs diet, purchased from General Biochemicals, Chagrin Falls, Ohio, was supplemented with vitamin C sufficient to supply guinea-pigs with 150 or 1000 mg/kg/day of ascorbic acid. For the rat studies, powdered Purina Rat Chow was supplemented with ascorbic acid so as to supply the animals with 1000 mg/kg/day. The diets were prepared by mixing in a rotary mixer for 3 h; fresh diet was prepared every 4 days. The animals were allowed food and water ad libitum. In these studies guinea-pigs and rats consumed 20–25 and 18–20 g of food per animal per day, respectively.

Male, Sprague-Dawley rats (200–250 g) or male, Hartley strain guinea-pigs (300–400 g) were used in our studies. When the guinea-pigs were received from the supplier, they were acclimated to our animal room for 10 days on a scorbutagenic diet which was supplemented with ascorbic acid so as to supply the animals with 150 mg/kg/day of the vitamin. This amount of ascorbic acid is approximately equivalent to the amount of vitamin C which guinea-pigs ingest when they are fed normal guinea-pig lab chow supplemented with a daily ration of lettuce. Rats were acclimated on standard Purina lab chow. The animals were then placed on the

[1] L. PAULING, Science *160*, 265 (1968).
[2] W. L. DALTON, J. Indiana State med. Ass. *55*, 1151 (1962).
[3] M. L. RUCCITELLI, J. Am. geriat. Soc. *20*, 34 (1972).
[4] L. PAULING, *Vitamin C and the Common Cold* (W. H. Freeman Co., San Francisco, California 1970).
[5] N. B. THOA, R. J. WURTMAN and J. AXELROD, Proc. Soc. exp. Biol. Med. *121*, 267 (1965).
[6] O. A. LOKOSHKO and L. D. LESNYKH, Ukr. Biokhem. Zh. *43*, 539 (1971).

Effect of ascorbic acid on tissue ascorbate and catecholamine levels in the rat and the guinea-pig[a]

Diet (mg ascorbate/ kg/day)	Organ	Rat			Guinea-Pig		
		Ascorbate (mg/100 g)	Norepinephrine (μg/g)	Dopamine (μg/g)	Ascorbate (mg/100 g)	Norepinephrine (μg/g)	Dopamine (μg/g)
0	Heart	10.2 ± 1.1 (9)	0.4 ± 0.11 (6)	—	—	—	—
	Brain	30.8 ± 6.5 (6)	0.23 ± 0.02 (6)	0.42 ± 0.06 (6)	—	—	—
150[b]	Heart	—	—	—	12.4 ± 0.8 (6)	2.10 ± 0.11 (6)	—
	Brain	—	—	—	19.3 ± 1.1 (6)	0.24 ± 0.01 (6)	0.53 ± 0.03 (6)
1000	Heart	13.6 ± 1.1 (9)	0.50 ± 0.04 (6)	—	13.9 ± 0.6 (6)	1.50 ± 0.11 (6)[c]	—
	Brain	31.4 ± 1.8 (6)	0.25 ± 0.01 (6)	0.32 ± 0.02 (6)	18.6 ± 1.6 (6)	0.24 ± 0.02 (6)	0.50 ± 0.05 (6)

[a] Figures in the table are the means + S.E.M. for the number of animals indicated in parentheses. [b] In our laboratory, guinea-pigs maintained on standard laboratory chow, supplemented with lettuce, receive approximately this amount of ascorbate daily. Therefore, the animals are considered to be normal controls with respect to ascorbate intake. [c] Statistically different from the heart level observed in animals ingesting 150 mg/kg/day of ascorbate; $P < 0.005$.

various test diets for 14 days; control animals were maintained on the diets used for acclimatization. On the 14th day the animals were killed and the hearts and brains were removed and freed of fat and connective tissue. The hearts and brains were homogenized in 20 and 4 volumes of 10% trichloracetic acid, respectively. Catecholamines in the clear protein-free supernatant were separated on alumina and Dowex columns as described previously[7], and the amines were quantified fluorometrically as described by LAVERTY and SHARMAN[8]. Separate aliquots of protein free supernatant were analyzed for ascorbic acid by the method of ROE and KUETHER[9].

Results. The results of the experiments are presented in the Table. Norepinephrine and dopamine levels in the brains of guinea-pigs fed 1000 mg ascorbate/kg/day for 13 days were not significantly different from the levels observed in animals which had received a basal supplement of 150 mg ascorbate/kg/day for the same amount of time. On the other hand, guinea-pigs which had been given the high dose of vitamin C contained heart levels of norepinephrine which were about 30% less ($P < 0.005$) than observed in animals on a basal dietary intake of ascorbate. In contrast to these findings in the guinea-pig, the catecholamine levels in the hearts and brains of rats remained unaltered regardless of their dietary intake of ascorbic acid. It should be noted that the decrease in heart norepinephrine observed in guinea-pigs on a high dietary intake of ascorbic acid was not accompanied by any alteration in the level of heart ascorbate.

Discussion. When guinea-pigs are administered 1000 mg/kg/day of ascorbic acid, one observes a 30% decrease in heart norepinephrine, whereas the brain level of the amine remains unchanged. An effect of vitamin C status on heart norepinephrine has been reported by others. THOA et al.[5] showed that scorbutic guinea-pigs have a reduced level of endogenous heart norepinephrine as well as a reduced ability of the heart to take up and bind the catecholamine. In guinea-pigs with hypovitaminosis C, norepinephrine levels are the same as in normal animals[6].

The mechanism by which a high dietary intake of ascorbic acid lowers heart norepinephrine is not clear. It would appear that the level of norepinephrine in the heart is not associated with the concentration of ascorbate in that organ since we have observed no differences in heart ascorbate concentration regardless of the dietary intake of the vitamin, and others have observed similar decreases in norepinephrine in scorbutic animals which presumably have less heart ascorbate than normals[5]. Under conditions in which one might expect to see a rise in heart ascorbate (10 min following a single 500 mg/kg dose of ascorbic acid, i.p.), IZQUIERDO and JOFRE[10] could observe no effect on heart norepinephrine concentration. It is possible that the levels of vitamin C in various anatomical regions of the heart were altered by our ascorbate dosing regimen but were not reflected by an alteration in the whole heart level of ascorbic acid. The mechanisms for the storage and uptake of norepinephrine by the heart are known to be influenced by sodium, potassium and calcium[11], and ASKARI et al.[12] have suggested that vitamin C may have a role in the active transport of sodium and potassium in certain tissues. Thus it is possible that in guinea-pigs fed relatively high levels of ascorbic acid, the vitamin may affect heart norepinephrine levels indirectly as a result of its effect on the sodium-potassium pump.

These findings suggest that the effect of a high dietary intake of ascorbic acid on heart norepinephrine is significant in an animal species such as the guinea-pig which cannot synthesize vitamin C. However, in the rat which is able to carry out the biosynthesis of ascorbic acid, high oral doses of ascorbate had no effect on heart norepinephrine[13].

Zusammenfassung. Längerdauernde Zufuhr hoher Dosen von Ascorbinsäure (1000 mg/kg) senkt bei Meerschweinchen, nicht aber bei Ratten, den Noradrenalin-Spiegel im Herzen signifikant. Hingegen wird bei beiden Tierspezies der Noradrenalin-Spiegel im Hirn nicht verändert.

T. DASHMAN, D. HORST, G. BAUTZ and J. J. KAMM

Research Division, Hoffmann-La Roche Inc., Nutley, (New Jersey 07110, USA), 27 February 1973.

[7] W. D. HORST and J. JESTER, Biochem. Pharmac. *20*, 2633 (1971).
[8] R. LAVERTY and D. F. SHARMAN, Br. J. Pharmac. *24*, 538 (1965).
[9] J. H. ROE and C. A. KUETHER, J. biol. Chem. *147*, 399 (1943).
[10] J. A. IZQUIERDO and I. J. JOFRE, J. Pharm. Pharmac. *17*, 391 (1965).
[11] W. D. HORST, I. J. KOPIN and E. R. RAMEY, Am. J. Physiol. *215*, 817 (1968).
[12] A. ASKARI, M. FREY, J. R. PITTS and S. N. RAO, 5th Int. Congress Pharm. (San Fransisco, California, 1972) Abstracts of Volunteer Papers, p. 11.
[13] The authors wish to thank Mr. G. MATUSIK and Mrs. HALYNA HOLOWASCHENKO for excellent technical assistance and Mrs. MARGARET ALTHOFF for her assistance in the preparation of the manuscript.