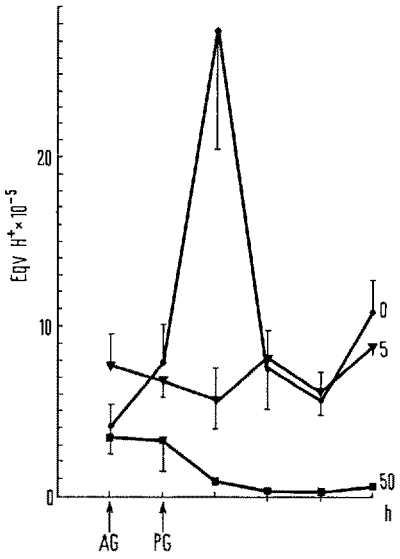
decarboxylase activity was determined by estimating the $^{14}CO_2$ released[18]. All assays were made in duplicate and the enzyme activities were expressed as nanomoles CO_2 formed per mg and hour. The results were corrected for non-enzymatic decarboxylation by incubating identical samples with 1-^{14}C-D-histidine ($4 \times 10^{-4} M$, 1.3 mC/mmol, New England Nuclear) instead of 1-^{14}C-L-histidine, or by using boiled tissue extracts incubated with 1-^{14}C-L-histidine. Usually, both types of blanks were run with each series of determinations. Gastric secretion was studied in Wistar rats, weighing 300–400 g fitted with chronic gastric fistulas[19]. For the collection of gastric juice the fistula rats were restrained in Bollman-type cages. The stomach was rinsed through the fistula with 0.9% warm saline until the return was clear. 10 ml 0.9% saline were given s.c. to replace fluid loss during collection. When the fistula had drained freely for 1 h, 1 h portions of gastric juice were collected. Usually, basal secretion was collected for 2 h, after which the injections were given and the collection continued. The volume of the gastric juice was measured and the acid output determined by titration with 0.02 N sodium hydroxide, using phenolphtalein as indicator. Acid output was expressed as equivalents per hour.



Inhibiton of pentagastrin-induced stimulation of gastric acid secretion by SC-15396 in chronic fistula rats. PG, pentagastrin, 250 µg/kg; AG, SC-15396, 'anti-gastrin', 5 or 50 mg/kg. The same group of rats (6 animals) was used througout. Mean ± S.E.M.

Effect of SC-15396 on gastric histidine decarboxylase activity in normal and antrectomized rats

Treatment	Histidine decarboxylase activity nmoles × 10^{-3}/mg/h, means ± S.E.M. (*n*) normal		antrectomy	
DMSO (5 ml/kg), 3 h	6.9 ± 2.4	(4)		
DMSO (5 ml/kg), 6 h	5.8 ± 1 2	(8)	2.6 ± 1.4	(4)
SC-15396, 3 h	4.4 ± 1.7	(5)		
SC-15396, 6 h	15.4 ± 2.9	(8)	1.9 ± 0.9	(5)

In a dose of 5 mg/kg, SC-15396 prevented the stimulatory effect of a maximal dose of pentagastrin (250 µg/kg); a larger dose of SC-15396 (50 mg/kg) was required to abolish basal acid secretion (Figure 1). 6 h after administration of SC-15396 (50 mg/kg) to normal, fasted, male Wistar rats (150–200 g), the gastric histidine decarboxylase activity was markedly increased as compared with controls (Table). Antrectomy, performed as described in detail elsewhere[5], prevented the enzyme-activating effect of SC-15396 (Table). The results indicate that the enzyme-activating effect of SC-15396 is mediated by some antral agent, possibly gastrin, which is released as a result of the inhibition of acid secretion. This gives further support to the hypothesis that the gastric histidine decarboxylase activity is regulated by endogenous antral gastrin and that a feed-back control exists between gastrin release and acid secretion[5,6]. Not only does a lowered antral pH inhibit gastrin release but elevated antral pH seems to be very effective in stimulating gastrin release[20].

Zusammenfassung. 2-Phenyl-2(2-Pyridyl)-Thioacetamide (SC-15396) ist ein kräftiger Inhibitor von sowohl basaler als auch stimulierter gastrischer Säuresekretion und aktiviert die Histidindecarboxylase in der Magenschleimhaut. Die enzymaktivierende Wirkung von SC-15396 wird durch Resektion des Antrums verhindert. Daraus kann geschlossen werden, dass die durch SC-15396 hervorgerufene Enzymaktivierung eine Folge von erhöhter Freisetzung von antralem Gastrin ist.

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Protective Effect of Flavonoids on the Collagen of Lathyritic Rats

The beneficial action of flavonoids in the maintenance or restoration of the normal integrity and permeability of the vessel wall is already known[1]. Various mechanisms have been suggested to explain such an effect, and among these it is possible to consider the action of the flavonoids on collagen.

Since it is possible to modify artificially the normal structure of collagen through the administration of lathyrogenic substances, producing an increase of soluble collagen by a block of the formation of cross-linkages[2], we planned to study the protective effect of flavonoids [*O*-(β-hydroxyethyl)-rutosides (HR) and (+)-catechin ((+)-C)][3] in rats treated by β, β′-iminodipropionitrile (IDPN) and aminoacetonitrile (AAN).

Bearing in mind that the lathyrogens produce some changes in the vascular system[4–7] and that flavonoids

mainly act at the vascular level, we undertook our research on the aorta of normal and treated animals, supported also by the protective action of HR on the retinal microvascular changes produced by IDPN, observed by PATERSON and HEATH[8].

Methods. In the present research, aminoacetonitrile (AAN) and β, β'-iminodipropionitrile (IDPN) were used to produce the lathyrism, in such a way as to obtain the typical symptoms, in the first case, of osteolathyrism[4,7] and, in the second case, of the 'ECC syndrome', the characteristics of which are very similar to, although not identical with, those of neurolathyrism[9].

The observations were carried out on the aorta, the wall of which can be affected by the action of the lathyrogens. Operating, however, on adult animals rather than on newborn or recently weaned ones, efforts were made to avoid[10] the serious angiolathyrism which, through the severity of the anatomical lesions (dissecting aneurysm of the aorta) can bring about a situation which is unfavourable for the identification of factors primarily modified by the experimental action. Sprague-Dawley rats about 3 months old were used at the beginning of the experiment; they were subdivided into groups of six. Three series of experiments were carried out which provided for various types of lesion induced by lathyrogenic substances and various types of possible protection with flavonoids (for details see Table).

Each experiment was conducted for a period of 33 days, with administration of flavonoids for the whole time and of the lathyrogens from the 6th to the 13th day, the doses of both also being indicated in the Table. All the animals were killed by decapitation and a careful dissection of the arch of the aorta and the whole of the thoracic aorta was effected. The aorta of each group of animals were collected together and utilized for the extraction and the dosage of the neutro-soluble collagen in accordance with the method of GROSS[11] dosing hydroxyproline in the extracts as a test of the presence of the collagen extracted. The data were tested by the analysis of the variance.

Results. From the results set out in the Table, an increase in the soluble fraction of collagen in the animals treated with the lathyrogens can be noted ($P < 0.05$). This indicates that, in the treatment with either IDPN or with AAN, a modification in the structure of the collagen was found. Such modifications are presumably of a different type for each of the two lathyrogens; in fact, while after the administration of AAN, 90% of the neutrosoluble collagen is already extracted in the first extraction, this does not take place in the treatment with IDPN. ($P < 0.01$).

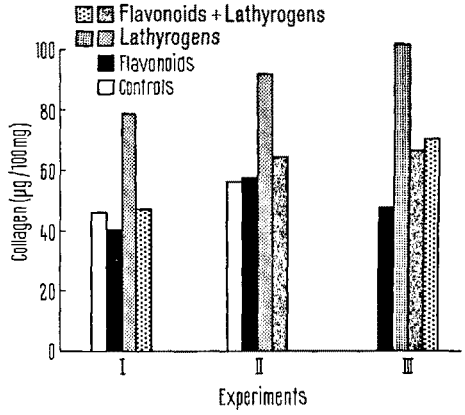
The administration of either HR or (+)-C in the course of the treatment of the rats with IDPN reduces the abovementioned modifications: the neutrosoluble collagen was produced in quantity equal to that found in the control animals. The administration of HR or of (+)-C during the treatment with AAN does not bring the soluble collagen content back to normal values but it reduces it to a consistent degree, so as to suggest a protective action. Since the damage caused by the AAN appears to be more serious than that experienced with IDPN, it can be supposed that to prevent it may prove more difficult.

Discussion. The complex of the reported effects of lathyrogens is of particular significance, because it also confirms the existence of an anomaly in the aggregation of the tropocollagen in the vessel walls, independent of the appearance of a severe angiolathyrism[10].

With regard to the mechanism whereby this action of the flavonoids on the aorta of lathyritic rats is explained, it appears to us possible that the flavonoids may interfere with the action of the lathyrogen at the level of the collagen fibrils. It is known that for some lathyrogens[2] there is a primary action on the structure of the collagen, in the

No. of groups		Substances administered	Neutrosoluble collagen/100 mg of fresh tissue (mg) 1st extraction	2nd extraction	Total
I. Experiment	1	–	32.3	13.9	46.2
	2	HR	33.1	6.6	39.8
	3	IDPN	20.2	58.8	79.5
	4	HR and IDPN	26.9	20.9	47.8
II. Experiment	5	–	39.3	16.9	56.2
	6	(+)-C	22.8	34.9	57.7
	7	IDPN	22.1	70.0	92.1
	8	(+)-C and IDPN	29.9	34.3	64.2
III. Experiment	9	(+)-C or HR	40.5	6.3	46.8
	10	AAN	91.3	10.4	101.7
	11	(+)-C and AAN	54.8	11.4	66.2
	12	HR and AAN	58.2	12.0	70.2

Groups 1 and 5: Controls. Administration of solvents only. Groups 3 and 7: Administration of IDPN s.c. (commercial preparation diluted 1:1 with physiological solution) in a daily dose of 25 mg/100 g of weight. Group 10: Treated with AAN orally with small gastric bougie, in a daily dose of 10 mg/100 g of weight, in 1% solution in H_2O. Group 2: Treatment with HR administered s.c. in a daily dose of 50 mg/100 g of weight (10% solution). Group 6: Treated with (+)-C given orally in a daily dose of 10 mg/100 g of weight (solution 1% in H_2O). Group 9: Treated with HR or with (+)-C according to the methods indicated for groups 2 and 6. Group 4: Administration of HR as indicated for group 2 and simultaneously of IDPN as stated for groups 3 and 7. Group 8: Treated with (+)-C as indicated for group 6 and simultaneously with IDPN as described for groups 3 and 7. Group 11: Treatment with (+)-C as shown for group 6 and simultaneously with AAN as indicated for group 10. Group 12: Treatment with HR as for group 2 and simultaneously administration of AAN as stated for group 10.



Variations in the neutrosoluble collagen content of aorta of lathyritic rats. For details see the legend of the Table.

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sense that they intervene at the level of the formation of cross-linkages between the fibrils. They inhibit the lysine-oxydase which, oxidizing the amine group in some of the lysine residues, creates the prior condition for the formation of the cross-linkages. A reduced formation of these linkages leads to an increase in the fragility of the connective tissue and can explain the various manifestations of lathyrism due to an abnormal structure of the connective tissue itself, including the vascular changes.

In the light of these facts, the action of the two flavonoids in question, tending to maintain the level of the soluble collagen at values approximating to normality, appears to signify a protection of the lysine-oxydase against the effect of the lathyrogens. It can be suggested as a hypothesis that IDPN and AAN may block the enzyme, acting directly on the active areas or complexing the Cu^{++}ion, which is indispensable for the oxidation of the amino group of the lysine. If, in fact, either the flavonoids or the aminonitriles may complex the Cu^{++} ion[12], the positive action could be due to the fact that they interfere in some way in the distribution of the Cu^{++} ion between the aminonitriles and the lysine-oxydase.

If, on the other hand, the lathyrogens inhibit the enzyme, acting at the level of the active areas, one might consider as a second hypothesis the removal, through the agency of the flavonoids, of the lathryrogens, which, once blocked, would leave the lysine-oxydase free to pursue its normal function.

Riassunto. È stato determinato il collagene neutro solubile dell'aorta in ratti trattati con latirogeni (*β*, *β*-iminodiproprionitrile, aminoacetonitrile) e flavonoidi [*O*-(*β*-idrossietil)rutosidi o (+)-catechina]. Il contenuto in collagene solubile è aumentato negli animali trattati con i latirogeni, mentre non risulta modificato qualora i latirogeni vengano somministrati ad animali trattati con flavonoidi.

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L-DOPA Treatment in Parkinson's Disease: Effect on Dopamine and Related Substances in Discrete Brain Regions

It is well established that in Parkinson's disease the concentrations of dopamine (DA) and its metabolite homovanillic acid (HVA) are greatly decreased in the striatum (= caudate nucleus and putamen), substantia nigra and globus pallidus[1]. Based on these findings L-3,4-dihydroxyphenylalanine (L-DOPA), DA's immediate precursor, was successfully introduced in the treatment of Parkinson's disease[2–4]. The present study reports on the metabolic fate of L-DOPA in the brains of Parkinsonian patients who chronically received large daily doses (2–6 g) of this drug until death. It was thought that such an investigation would contribute to our understanding of the mechanisms through which L-DOPA exerts its effects in patients with Parkinson's disease.

Results and discussion. The brains of control patients (9 cases) and patients with Parkinson's disease (3 non-DOPA treated cases, 4 cases with L-DOPA treatment) were obtained and processed as previously described[5]. The following substances were estimated[6] in several discrete brain regions: DOPA, 3-*O*-methyl-DOPA, DA and HVA. The results of the study are summarized in the Table.

In control and non-DOPA treated patients neither DOPA nor 3-*O*-methyl-DOPA could be detected in any examined brain area. In these groups of patients the values for DA and HVA in the caudate nucleus and putamen agree well with earlier reports[7–10] illustrating the striatal DA deficiency as characteristic of Parkinson's disease. In contrast, in DOPA treated patients the DA concentrations in the caudate nucleus and putamen were 4–8 times higher than in the non-DOPA treated group of the present and previously reported studies. In the caudate nucleus of the DOPA-treated groups the mean DA concentration (2.2 μg/g) approached control values. The higher levels of striatal DA in the DOPA-treated group are most probably related to L-DOPA's transfromation to DA; they cannot be explained by assuming that this group of patients had a milder degree of degeneration of the nigro-striatal DA neurons than the non-DOPA-treated group. This follows from our observations[11] that a) in both groups of patients the depigmentation of the substantia nigra and the decrease in striatal L-DOPA decarboxylase activity were of the same magnitude, and b) the levels of DA in the striatum of the DOPA-treated patients were directly related to the administered dose of L-DOPA (which varied from 2–6 g daily) and the time interval between the last dose of the drug and death of the patient (4–24 h). This latter observations also help to explain the large scatter of the values for DA (and the other substances estimated; see Table) in this group of patients, which in most instances precluded a statistical evaluation of the results obtained; from the point of view of statistical analysis, the patient material that constituted the DOPA-treated group was by necessity non-homogeneous.

In analogy to DA, the mean concentrations of HVA in the caudate and putamen in the DOPA-treated group were 8–20 times higher (8–11 μg/g) than in the non-DOPA treated Parkinsonian patients, being about twice as high as the control values. In addition, substantial though lower amounts of HVA (3–5 μg/g) were also detected in many extra-striatal brain regions; in these regions no DA was detected as result of treatment with L-DOPA. This finding shows that in the extra-striatal regions there is formation of, but no significant storage capacity for, DA. The preferential accumulation of DA and HVA in the Parkinsonian striatum indicates that a certain degree of regional selectivity and specificity of L-DOPA's metabolic transformation is still preserved in the brain of the diseased patients. However, nothing certain can at present be said about the cellular structures, in the striatum or other regions of the Parkinsonian brain, in which the therapeutically administered L-DOPA is metabolized. It is possible that, besides the still preserved DA neurons, the serotonin and noradrenaline neurons are involved[12].