TABLE 1. THE ACTION OF DOPAMINE AND NORADRENALINE ON THE BIOSYNTHESIS OF PGE_2 AND PGE_3 IN HOMOGENATES OF PARAVERTEBRAL GANGLIA

	Basal	Dopamine	Dopamine + Phentol-amine	Dopamine + Propronolol	Noradren-aline	Noradren-aline + Phentol-	Noradren-aline + Propro-nolol
PGE_2	4·1	9·8	3·9	9·6	3·8	3·9	3·8
PGE_3	3·6	3·7	3·4	3·6	8·0	7·7	3·5

The results (means of five experiments) as expressed as nanomoles of prostaglandin synthesized per 10 mg protein per hour. The drugs were added at a concentration of 10^{-7} M.

In a thoroughly teased preparation of brown fat, or free fat cells prepared by collagenase[7] dopamine and diethyl dithiocarbamate + iproniazid, alone or together with DFP had no action on basal respiration. This is presumably due to disruption and loss of ganglia. In such preparations, as is well known, noradrenaline stimulated respiration by acting directly on the β-receptors of adipocytes.

Acknowledgement—We are indebted to Professor C. W. M. Adams for his continuous encouragement.

Department of Pathology,
Guy's Hospital Medical School,
London, S.E. 1

Y. H. ABDULLA
ELIZABETH MCFARLANE

REFERENCES

1. D. M. DERRY, E. SCHÖNBAWM and G. STEINER.
2. W. H. COTTLE, in *Brown Adipose Tissue* (Ed. O. LINDBERG), Elsevier, New York (1970).
3. D. A. VAN DORP, G. H. JOUVENAZ and C. D. STRUIJK, *Biochim. biophys. Acta* **137**, 396 (1967).
4. D. HULANICKA, J. ERWIN and K. BLOCK, *J. biol. Chem.* **339**, 2778 (1964).
5. Y. H. ABDULLA and E. MCFARLANE, *Biochem. Pharmac.* **20**, 1726 (1971).
6. A. M. CARLSSON, M. LINDQUIST, K. FUXE and T. HOKFELT, *J. Pharm. Pharmac.* **18**, 60 (1966).
7. J. N. FAIN, N. REED and R. SAPERSTEIN, *J. biol. Chem.* **242**, 1887 (1967).

Biochemical Pharmacology, Vol. 21, pp. 594–600. Pergamon Press, 1972. Printed in Great Britain

The effect of (+)-catechin on the hepatic level of ATP and the lipid content of liver during experimental steatosis

(*Received* 8 *July* 1971; *accepted* 8 *October* 1971)

THE BIOCHEMICAL effect of the bioflavonoids is unknown. The common name "vitamin P", often used to designate them, has been strongly criticized. Many authors think that several reported physiological actions of the flavonoids could be ascribed to the ascorbic acid content of the preparations used.

Our attention was drawn to a possible role of flavonoids in the biosynthesis of ATP by the observation of Teras.[1–3] This author has reported that purified preparations of rutin and catechins uncoupled oxidative phosphorylation and stimulated the activity of ATP-ase in rats and in mitochondria isolated from their livers.

In our experiments with a chemically pure preparation of (+)-catechin[4] we have found that the

effect of this compound on the hepatic level of ATP is more complicated. When a daily dose of 25 mg/kg of this compound was administered subcutaneously for 2 weeks to normal adult rats, the concentration of ATP in the liver was diminished. But when the dose of (+)-catechin was doubled, the level of ATP in the liver was significantly increased ($P < 0.001$).

These observations led us to study the following two problems. (1) Would the relatively high doses of (+)-catechin act similarly in rats intoxicated with drugs which markedly lower the hepatic level of ATP? (2) If it is so, would the treatment with (+)-catechin diminish the lipid content of the liver in rats with experimental hepatic steatosis? Indeed, Hyams and Isselbacher[5] have shown in 1964 that the accumulation of fat in the liver of rats provoked by different toxic agents (carbon-tetrachloride, ethanol, azaserine, ethionine) is accompanied by a marked decrease in the concentration of ATP in this organ and that parenteral administration of ATP is accompanied by a significant decrease in the lipid content of the liver.

Our experiments, the results of which will be briefly expounded, have shown that one of the main biochemical effects of (+)-catechin may be the stimulation of the *in vivo* biosynthesis of ATP.

Materials and methods

Chemically pure (+)-catechin was kindly given by Zyma (Nyon, Switzerland). In all experiments, adult female Wistar rats of about 200 g were used.

Two toxic agents capable of lowering the hepatic level of ATP were used: malonic acid administered subcutaneously for 2 weeks at a daily dose of 1500 mg/kg and D-L-ethionine introduced by gastric tube for 7 days at a daily dose of 200 mg/kg. From the beginning of intoxication, the rats received a daily subcutaneous injection of 45 mg/kg of (+)-catechin in 2 ml saline. To the control intoxicated rats, 2 ml saline was administered daily.

The hepatic steatosis was provoked in three different ways: by a hypoproteinic diet and by administration of either orotic acid or ethanol.

The steatogenic diet administered for 2 weeks contained 10% of casein as the only source of protein, 30% of fat, 55% of sucrose and 5% of a salt mixture.

For the intoxication by orotic acid, the technique of von Euler *et al.*[6] was employed: the rats were fed a diet containing 80% sucrose, 12% casein, 3% fat, 1% orotic acid and 4% salt mixture for 2 weeks.

Ethanol, diluted three times with water was administered intraperitoneally twice a day for 1 week. The dose of each injection was 7 ml/kg.

In the three last series, the (+)-catechin was administered, from the beginning of the experiments, either subcutaneously (50 mg/kg/day) or by gastric tube (200 mg/kg/day). The control rats received the corresponding volume of saline.

At the end of the experiments, the rats were killed by decapitation and the liver quickly removed in ice. The following quantitative determinations were performed: ATP by fire-fly method,[7] NAD and NADH by the enzymic method of Jedekein and Weinhouse,[8] total liver lipids by extraction in micro-kumagawa and weight of the dried extract. Liver slices were also removed for histological examination.

Results

As shown in Table 1, in rats intoxicated with either malonic acid or ethionine, the hepatic level of ATP is markedly diminished as compared to the normal mean. In contrast, in rats intoxicated and at the same time treated with (+)-catechin, the concentration of ATP in the liver is higher, than in the intoxicated and non-treated animals. The differences are statistically highly significant.

In the rats fed the low protein high fat diet, we found, besides the fatty liver, a marked decrease in the level of ATP and the accumulation of NADH leading to a diminished NAD/NADH ratio. All these biochemical troubles were significantly corrected in the animals having received a daily subcutaneous injection of 50 mg/kg of (+)-catechin (Table 2).

The biochemical anomalies in the liver of rats intoxicated with orotic acid were similar to those observed in rats fed a low protein high fat diet. Here again, the effect of parenterally administered (+)-catechin was clear on all the parameters studied, except the variations of bodyweight (Table 3).

In the rats intoxicated with orotic acid, the beneficial effect of (+)-catechin administered orally at the daily dose of 200 mg was similar to that observed following parenteral administration of the flavonoid (Table 4).

Particularly interesting was the study of the effects of (+)-catechin on the liver metabolism in rats intoxicated with ethanol. The hepatic biochemical disorders provoked by high doses of ethanol are indeed well known and this fact may furnish a very useful base for the discussion of the mechanism of action of the (+)-catechin.

As in our former experiments, parenteral administration of (+)-catechin to rats intoxicated with ethanol improved all the metabolic troubles studied, but again did not influence the body weight (Table 5).

Similar effects of (+)-catechin were observed, when this flavonoid was administered orally (Table 6).

TABLE 1. EFFECT OF ADMINISTRATION OF (+)-CATECHIN ON THE HEPATIC LEVEL OF ATP IN RATS INTOXICATED WITH MALONIC ACID OR WITH D-L-ETHIONINE

	Hepatic level of ATP (μmoles per 100 tissue, wetweight)	
Normal rats ($n = 12$)	173 ± 3·9	
Rats intoxicated with malonic acid ($n = 15$)	102 ± 4·8	
Rats intoxicated with malonic acid and treated with parenterally administered (+)-catechin, 45 mg/kg/day ($n = 15$)	196 ± 15·2	$P < 0·001$
Rats intoxicated with D-L ethionine ($n = 15$)	106 ± 3·7	
Rats intoxicated with D-L ethionine and treated with parenterally administered (+)-catechin ($n = 15$)	126 ± 5·63	$P < 0·001$

TABLE 2. EFFECT OF (+)-CATECHIN ON THE METABOLIC TROUBLES IN THE LIVER OF RATS FED A LOW PROTEIN HIGH FAT DIET

Parameters studied	Rats fed a low protein high fat diet ($n = 12$)		Rats fed the same diet treated with a daily subcutaneous injection of 50 mg/kg of (+)-catechin ($n = 12$)
Variation of weight (% of the initial weight)	+ 5·0 ± 0·8	$P > 0·10$	+ 5·1 ± 0·28
ATP (μmoles/100 g hepatic tissue w.w.)	105 ± 12·2	$P < 0·05$	141 ± 8·77
NAD (μg/g of liver)	395 ± 12·3	$P > 0·05$	403 ± 11
NADH (μg/g of liver	451 ± 7·8	$P < 0·001$	406 ± 6·6
NAD/NADH	0·86 ± 0·028	$P < 0·001$	0·99 ± 0·029
Total lipid content (g/100 g)	5·58 ± 0·25	$P < 0·01$	4·55 ± 0·35

Normal means ($n = 12$): ATP 173 ± 3·9. NAD 373 ± 7·1. NADH 261 ± 10·3. NAD/NADH 1·45 ± 0·049. Liver lipids 3·8 ± 0·071.

TABLE. 3. EFFECT OF PARENTERAL ADMINISTRATION OF (+)-CATECHIN (50 mg/kg/day) ON THE METABOLIC TROUBLES OF LIVER OF RATS INTOXICATED WITH OROTIC ACID

Parameters studied	Rats intoxicated with orotic acid ($n = 12$)		Rats intoxicated with orotic acid and treated with a daily subcutaneous injection of 50 mg/kg of (+)-catechin ($n = 12$)
Variation of weight (% of initial weight)	$-4{\cdot}9 \pm 1{\cdot}2$	$P > 0{\cdot}10$	$-2{\cdot}4 \pm 0{\cdot}86$
ATP (μmoles/100 g)	$88{\cdot}2 \pm 6{\cdot}4$	$P < 0{\cdot}01$	$116 \pm 6{\cdot}8$
NAD (μg/g)	$320 \pm 15{\cdot}5$	$P < 0{\cdot}01$	$372 \pm 10{\cdot}6$
NADH (μg/g)	$468 \pm 22{\cdot}9$	$P < 0{\cdot}05$	$400 \pm 18{\cdot}8$
NAD/NADH	$0{\cdot}70 \pm 0{\cdot}055$	$P < 0{\cdot}01$	$0{\cdot}95 \pm 0{\cdot}054$
Total lipid content g/100 g	$10{\cdot}2 \pm 0{\cdot}59$	$P < 0{\cdot}001$	$6{\cdot}4 \pm 1{\cdot}3$

TABLE 4. EFFECT OF ORAL ADMINISTRATION OF (+)-CATECHIN (200 mg/kg/day) ON THE METABOLIC TROUBLES OF LIVER OF RATS INTOXICATED WITH OROTIC ACID

Parameters studied	Rats intoxicated with orotic acid ($n = 12$)		Rats intoxicated with orotic acid and treated with oral administration of (+)-catechin 200 mg/kg/day ($n = 12$)
Variation of weight (% of initial weight)	$-4 \pm 1{\cdot}1$	$P > 0{\cdot}10$	$-4{\cdot}0 \pm 0{\cdot}54$
ATP (μmoles/100 g)	$70 \pm 5{\cdot}0$	$P < 0{\cdot}001$	$112 \pm 6{\cdot}5$
NAD (μg/g)	$308 \pm 16{\cdot}1$	$P < 0{\cdot}01$	$414 \pm 13{\cdot}5$
NADH (μg/g)	$434 \pm 18{\cdot}8$	$P < 0{\cdot}05$	$376 \pm 16{\cdot}4$
NAD/NADH	$0{\cdot}71 \pm 0{\cdot}039$	$P < 0{\cdot}001$	$1{\cdot}08 \pm 0{\cdot}055$
Total lipids (g/100 g)	$9{\cdot}66 \pm 0{\cdot}64$	$P < 0{\cdot}02$	$6{\cdot}1 \pm 0{\cdot}8$

TABLE 5. EFFECT OF PARENTERAL ADMINISTRATION OF (+)-CATECHIN ON THE BIOCHEMICAL TROUBLES OF LIVER OF RATS INTOXICATED WITH ETHANOL

Parameters studied	Rats intoxicated with ethanol ($n = 12$)		Rats intoxicated with ethanol and treated with a daily subcutaneous injection of 50 mg/kg of (+)-catechin ($n = 12$)
Variation of weight (% of initial weight)	$-2{\cdot}7 \pm 0{\cdot}34$	$P > 0{\cdot}05$	$-0{\cdot}5 \pm 0{\cdot}77$
ATP (μmoles/100 g)	$111 \pm 3{\cdot}83$	$P < 0{\cdot}001$	$196 \pm 8{\cdot}7$
NAD (μg/g)	$378 \pm 10{\cdot}03$	$P < 0{\cdot}05$	$382 \pm 6{\cdot}8$
NADH (μg/g)	$419 \pm 9{\cdot}39$	$P < 0{\cdot}001$	$339 \pm 8{\cdot}2$
NAD/NADH	$0{\cdot}91 \pm 0{\cdot}030$	$P < 0{\cdot}001$	$1{\cdot}12 \pm 0{\cdot}029$
Total lipids (g/100 g)	$7{\cdot}0 \pm 0{\cdot}19$	$P < 0{\cdot}001$	$5{\cdot}3 \pm 0{\cdot}18$

TABLE 6. EFFECT OF ORAL ADMINISTRATION OF (+)-CATECHIN ON THE BIOCHEMICAL TROUBLES OF LIVER OF RATS INTOXICATED WITH ETHANOL

Parameters studied	Rats intoxicated with ethanol ($n = 12$)		Rats intoxicated with ethanol and treated with a daily oral dose of 200 mg/kg of (+)-catechin($n = 12$)
Variation of weight (% of initial weight)	$-4{\cdot}3 \pm 0{\cdot}47$	$P > 0{\cdot}05$	$-3{\cdot}6 \pm 0{\cdot}30$
ATP (μmoles/100 g	$106 \pm 5{\cdot}19$	$P < 0{\cdot}02$	$140 \pm 14{\cdot}7$
NAD (μg/g)	$325 \pm 9{\cdot}9$	$P < 0{\cdot}01$	$361 \pm 8{\cdot}4$
NADH (μg/g)	$427 \pm 7{\cdot}72$	$P < 0{\cdot}001$	$337 \pm 9{\cdot}2$
NAD/NADH	$0{\cdot}77 \pm 0{\cdot}031$	$P < 0{\cdot}001$	$1{\cdot}09 \pm 0{\cdot}023$
Total lipids (g/100 g)	$6{\cdot}6 \pm 0{\cdot}14$	$P < 0{\cdot}001$	$4{\cdot}5 \pm 0{\cdot}26$

The decrease in the accumulation of lipids in the liver following administration of (+)-catechin was also histologically confirmed in all our three series of experimental hepatic steatosis.

Discussion

Our experiments confirm our early observations showing the stimulating effect of (+)-catechin on the biosynthesis of ATP in the liver. They also confirm the findings of Hyams and Isselbacher[5] establishing a reverse correlation between the hepatic concentration of ATP and the accumulation of lipids in this organ in various experimental conditions. The effect of the flavonoid studied by us on the hepatic level of ATP is remarkably constant. We have observed it indeed in the normal rats as well as in rats intoxicated with four different toxic agents capable of lowering the level of this nucleotide in the liver.

In our three series of experiments on hepatic steatosis, a highly significant accumulation of NADH was noted leading to the marked decrease in the ratio NAD/NADH. These metabolic troubles were also significantly corrected by the administration of (+)-catechin.

This flavonoid exerted the same metabolic effect whether it was administered parenterally or orally.

Our experiments also clearly show that the improvement of the metabolic disorders in the liver by (+)-catechin was not secondary to an effect on the nutrition and the general condition of the animals. Indeed, in all our experiments, the variations of the body weight, the food consumption and the general aspect of the rats remained the same whether they were treated or not with (+)-catechin.

The simultaneous increase in the level of ATP on the one hand, and the decrease in the accumulation of NADH leading to an elevation of the NAD/NADH ratio on the other hand in the animals treated with (+)-catechin are particularly interesting, since they suggest the stimulation by the flavonoid of the formation of ATP by oxidative phosphorylation. In the respiratory chain, the redox couple NAD/NADH is indeed the first locus of the phosphorylation of ADP to ATP. Our findings seem therefore to confirm the conclusions of Fritz-Niggli.[9] This author reported that the oxidative phosphorylation in isolated liver mitochondria, uncoupled by bilirubin, was re-established to normal levels through the effect of a bioflavonoid, hydroxyethylrutoside.

Our findings in rats intoxicated with ethanol however showed that the problem is highly complicated.

The classical biochemical character of ethanol intoxication is the accumulation of NADH in the liver. This accumulation is the consequence of the oxidation of ethanol to acetaldehyde. The obligatory coenzyme of the alcohol-dehydrogenase catalyzing this reaction is NAD serving as acceptor of hydrogen.

The NADH formed must be located in the liquid phase of the hepatocytes where is also located the alcohol-dehydrogenase. The decrease in the hepatic accumulation of NADH following administration of (+)-catechin to rats intoxicated with ethanol cannot be associated with the mitochondrial redox couple NAD/NADH, since the mitochondrial membrane is not permeable to these two nucleotides. The reoxidation of NADH to NAD, indispensable to maintaining the dehydrogenation of the high doses of continuously administered ethanol, must therefore be performed in the cytoplasm. It may take place in this cellular compartment during the reduction of pyruvate to lactate, a reaction catalized by lactic dehydrogenase. The coenzyme is NADH, serving as donator of hydrogen. This last reaction may be a part of a biochemical defense mechanism during ethanol intoxication. As we have reported elsewhere,[10] during intoxication of rats with repeated high doses of ethanol, the hepatic level of lactate is very significantly increased. The administration of (+)-catechin could stimulate the glycolysis, source of the pyruvate. Therefore the increase in the hepatic level of ATP by this flavonoid might be explained, at least partly, by the formation of the nucleotide in the Embden–Meyerhoff pathway.

If the enhancement of the biosynthesis of ATP in the liver by (+)-catechin seems to be well established, the difficult problem of the biochemical mechanism of this effect can only be solved by future investigations.

In any case, the stimulating effect of (+)-catechin on the formation of ATP may be considered as a true vitaminic character capable of explaining some of the physiological actions of this flavonoid.

Summary

Parenteral or oral administration of chemically pure (+)-catechin increases the hepatic level of ATP in rats intoxicated with malonic acid or ethionine or in rats with liver steatosis provoked by a low protein high fat diet or by administration of orotic acid or ethanol. The accumulation of NADH and lipids in the liver tissue is also significantly lowered following treatment of the intoxicated rats with (+)-catechin. The biochemical mechanism of these effects is discussed.

Laboratoire de Recherches biochimiques,
Université 5 de Paris,
1, *rue Lacretelle Paris* 15°.

A. GAJDOS
M. GAJDOS-TÖRÖK
R. HORN

REFERENCES

1. L. TERAS, *Eesti, NSV Teabuste Akad. Toimetised* **14,** 135 (1965).
2. L. TERAS, *Eesti NSV Teabuste Akad. Toimetised* **15,** 587 (1966).
3. L. TERAS, *Biokimya Cal'nogo proizvodstva* **9,** 201 (1962).
4. A. GAJDOS, M. GAJDOS-TÖRÖK and R. HORN, *C.r. Soc. Biol.* **163,** 2089 (1969).
5. D. E. HYAMS and K. J. ISSELBACHER, *Nature, Lond.* **204,** 1196 (1964).
6. L. H. VON EULER, H. J. RUBIN and R. E. HANDSCHUMACHER, *J. biol. Chem.* **238,** 2464 (1963).
7. B. L. STREHLER and J. R. TOTTER, in *Glick Methods of Biochemical Analysis*, p. 341. Interscience, New York (1954).
8. L. A. JEDEKEIN and S. J. WEINHOUSE, *J. biol. Chem.* **213,** 271 (1955).
9. H. FRITZ-NIGGLI, *Med. Exp. (Basel)* **18,** 239 (1968).
10. A. GAJDOS et M. GAJDOS-TÖRÖK, *Biochim. Biophys. Acta* **215,** 550 (1970).