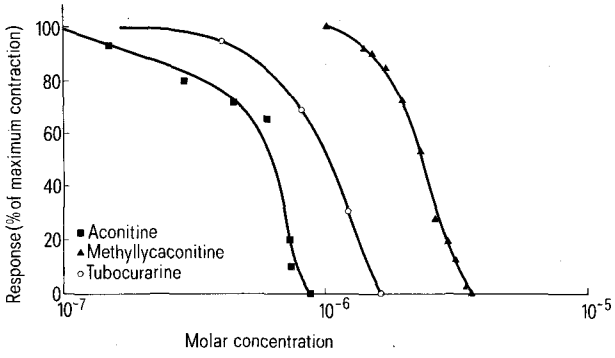
Concentrations of alkaloids of *D. brownii* producing 50% (ED_{50}) and 100% (ED_{100}) depression of the response of the rat phrenic nerve-diaphragm preparation

Fraction	Concentration* ED_{50} M/l	g/ml	ED_{100} M/l	g/ml
Aconitine hydrochloride	5.7×10^{-7}	3.9×10^{-7}	8.8×10^{-7}	6.0×10^{-7}
Tubocurarine chloride hydrochloride	1.0×10^{-6}	6.8×10^{-6}	1.6×10^{-6}	1.1×10^{-6}
Methyllycaconitine hydrochloride	2.3×10^{-6}	1.6×10^{-6}	3.6×10^{-6}	2.5×10^{-6}
Extract A**		3.0×10^{-4}		$\sim 6.0\times10^{-4}$
Extract B**		4.0×10^{-5}		$\sim 6.0\times10^{-5}$

* Final bath concentrations (regression values). ** Total methanol extract (after defatting with petroleum ether) (A), and mixture of water-insoluble tertiary bases (B). These fractions were suspended in Cremophor EL.



Dose-response for rat phrenic nerve-diaphragm.

good approximation the activity of the tertiary base fraction could be accounted for in terms of its MLA content. The toxicity of *D. brownii* thus appears to be very largely due to this alkaloid.

The dose-response curve of MLA seemed to parallel those of the reference compounds (figure), although regression analysis revealed a closer similarity to aconitine. The blockade by MLA was at least partially reversed by eserine, and the alkaloid could slowly be washed out on equilibration of the paralysed tissue with fresh physiological solution.

A number of preliminary electrophysiological and mechanical assays have also been performed using the sciatic nerve-sartorius muscle preparation of the frog, *Rana pipiens*. During nerve stimulation, ca. 10^{-7}M MLA produced a 50% inhibition of post-synaptically recorded action potentials within 20 sec. Complete muscle paralysis was achieved after 5 min. With ca. 10^{-8}M MLA the same level of inhibition was achieved after 50 sec, though total inhibition was not achieved at this concentration. The alkaloid could be washed out completely in both cases, but its action was only partially antagonised by 10^{-5}M eserine. Electrical conduction along the sciatic nerve, or over directly stimulated sartorius muscle was not affected by MLA at 10^{-7} M. Direct stimulation of the paralysed muscle elicited a normal contraction.

We conclude that a primary mode of action of MLA is by competitive blockade at nicotinic receptors, in substantial agreement with Dozortseva's findings[7], and the sometime clinical use of the hydroiodide salt of the alkaloid as a curare substitute[6], in the USSR. However, like aconitine, it may also affect sodium channels[11,12]. It is interesting that the neuro-muscular activity of MLA is essentially destroyed if the aromatic ester function is removed, for we found the parent alkaloid, lycoctonine **(1d)** to be essentially devoid of activity.

* To whom reprint requests should be addressed. Department of Chemistry.

1 We thank the University of Calgary, The National Research Council of Canada, and the Alberta Agriculture Department, for financial assistance.

2 R.F. Keeler, Llyodia *38*, 56 (1975).
3 V.N. Aiyar, M. Benn, Y.Y. Huang, J.M. Jacyno and A.J. Jones, Phytochemistry *17*, 1453 (1978).
4 F.N. Dzhakhangirov, I. Khamdamov and F.S. Sadritdinov, Dokl. Akad. Nauk. Uzbek. S.S.R., 32, (1976); Chem. Abstr. *85*, 103953x (1976).
5 I. Khamdamov, F.N. Dzhakhangirov and F.S. Sadritdinov, Dokl. Akad, Nauk. Uzbek. S.S.R., 37 (1975); Chem. Abstr. *84*, 84349r (1976).
6 I.A. Gubanov, Planta Medica *13*, 200 (1965).
7 P.M. Dozortseva, Farm Toxik. *22*, 34 (1959).
8 M.N. Mats, Rast. Resur. *8*, 249 (1972); Chem. Abstr. *77*, 79509 (1972).
9 E. Bülbring, Br. J. Pharmac. *1*, 38 (1946).
10 W.D.M. Paton, Br. J. Pharmac. *12*, 119 (1957).
11 W.A. Catterall, J. biol. Chem. *252*, 8669 (1977).
12 G.N. Moseyeva, A.P. Naumov, Y.A. Negulyaev and E.D. Nosyreva, Biochim. biophys. Acta *466*, 461 (1977).

A prostaglandin-like activity in small intestine and postirradiation gastrointestinal syndrome[1]

A. Borowska, S. Sierakowski, J. Maćkowiak and K. Wiśniewski

Department of Pharmacology, Institute of Pharmacology and Toxicology, Medical School, Białystok (Poland), 8 January 1979

Summary. A correlation between the postirradiation increase of the small intestine motility and the prostaglandin-like activity in this organ during gastrointestinal syndrome was observed. Indomethacin decreased the elevated motility of intestine and reduced the prostaglandin-like activity in this syndrome.

It was found recently that the increase of prostaglandin content in gastrointestinal tract which appears in such pathological conditions as colitis ulcerosa[2-4], thyroid medullar carcinoma[5] or cholera[6,7], induces diarrhea probably by stimulating the intestinal motility and reducing the epithelial water transport[8,9]. Also the therapeutic effect of diphenolic laxatives, like bisacodyl and phenophtalein, seems to be exerted by stimulation of prostaglandin synthesis[10].

The gastrointestinal syndrome which is manifested by

decrease of water and electrolyte imbibition, protein loss and significant stimulation of intestinal motility, appears to be one of the main causes of death in 40-45% irradiated animals in the first acute period of this disease[11].

Taking the above into account, it seemed to be of interest to observe the relationship between the disturbances of intestinal motility and prostaglandin-like activity in small intestine.

Material and methods. The experiments were carried out on male Swiss strain mice aged 3-7 months and weighing 25-30 g. The animals were exposed to 600 R in the following conditions: 160 kV, 20 mA with filters 0.5 mm Cu and 1 mm Al, FDS 50 cm, dose rate 44.2 R/min.

All the animals were divided into 2 groups: 1 group was used for studies of intestinal motility according to the classical method of Lowe and Faure[12]. The contrast was administrated per gastric tube and after 5 min the animals were decapitated and the position of contrast in the intestine was estimated. Results were expressed in per cent of length of the total intestine.

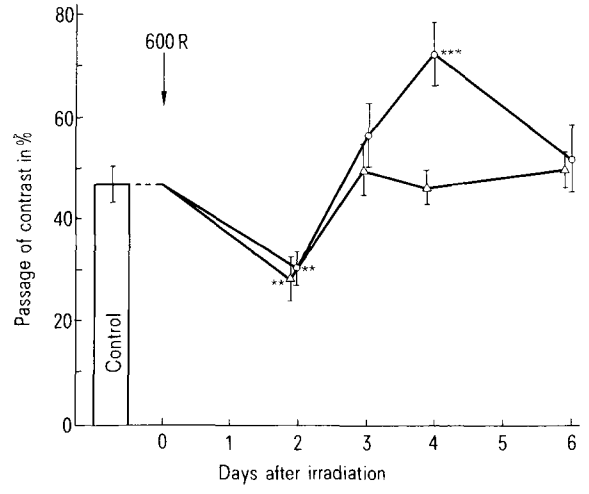


Fig. 1. The effect of irradiation (600 R, ○——○) and Indomethacin plus irradiation (△——△) on the small intestine motility of mouse. ** $p < 0.01$; *** $p < 0.001$.

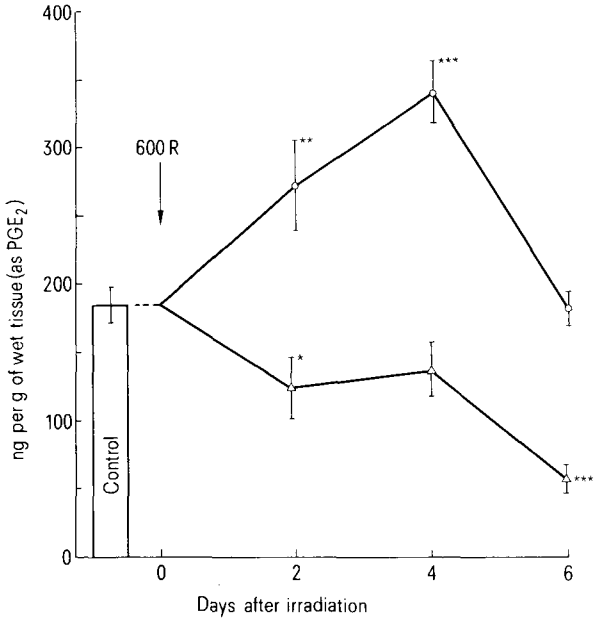


Fig. 2. The effect of irradiation (600 R, ○——○) and Indomethacin plus irradiation (△——△) on prostaglandin-like activity in the small intestine of mouse. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Animals of group 2 were killed by decapitation, small intestines were immediately excised and put into ice-cold physiological saline with the addition of Indomethacin (2 µg/ml) in order to prevent the prostaglandin synthesis during the experimental procedure[13]. The intestines were weighed, homogenized and the prostaglandin-like material was isolated according to the method of Unger, Stamford and Bennett[14]. The homogenates were mixed with absolute ethanol in the ratio 1:1 and incubated overnight at 4 °C, and then the total mixture was washed twice with petroleum ether. The alcohol-water layer was acidified with formic acid to pH below 3 and submitted to extraction with chloroform. The chloroform extract was evaporated at 30 °C and the excess of formic acid was removed by blowing with nitrogen (free of oxygen). The recovery of prostaglandin E_2, estimated by this method with the use of internal standards, was found to be in the range of 70-80%.

The prostaglandin-like activity was assayed on rat's stomach strips[15] superfused with tyrod solution which contained the mixture of antagonists: phenazolinum, atropinum sulfate, propranolol, Indomethacin and Regityne. The gas mixture consisting of 95% O_2 + 5% CO_2 was continuously bubbled through solution.

Some animals of groups 1 and 2 were treated with an inhibitor of prostaglandin biosynthesis - Indomethacin (Metindol 'Polfa') given per gastric tube in the suspension of arabic gum, 90 min before irradiation, in the dosis of 3 mg/kg and then twice a day in a dosis of 1 mg/kg.

The results were submitted to statistical analysis with the use of Student's t-test. Prostaglandin E_2 was kindly supplied by Dr J. Pike (Upjohn, Kalamozoo).

Results and discussion. It was found that the small intestine motility after the temporary decrease on the 2nd day after irradiation increased on the next days and reached the maximal level on the 4th day after irradiation. In this period, maximal enhancement of clinical symptoms was observed. The postirradiation increase of small intestine motility correlates with the increase of prostaglandin-like activity in this organ. Indomethacin prevents the postirradiation increase of small intestine motility and simultaneously decreases prostaglandin-like activity. Aspirin - another inhibitor of prostaglandin biosynthesis - exerts similar effect (unpublished observations).

The normalizing effect of prostaglandin biosynthesis inhibitors on some radiation-induced disturbances allows us to conclude that the stimulation of prostaglandin synthesis is responsible for the increase of the level of these substances in the irradiated tissues.

On the other hand, Eisen and Walker[17] observed postirradiation decrease of prostaglandin 15-OH dehydrogenase in the small intestine of mouse. This observation suggests that the decreased catabolism of prostaglandin may be a cause of these phenomena. It is of interest that these authors also observed an increase of prostaglandin-like activity in small intestine of irradiated animals, but this effect was statistically insignificant[18,19].

It seems possible that their studies were performed in too early period after irradiation - before manifestation of gastrointestinal disturbances in radiation conditions used in their experiments.

The existence of correlation between radiation-induced increase of intestinal motility and prostaglandin-like activity seems to elucidate the mechanism of beneficial action of inhibitors of prostaglandin biosynthesis in the case of patients suffering from gastrointestinal disorders induced by radiotherapy[20].

1 Acknowledgments. The work was supported by the Polish Academy of Sciences.
2 S.R. Gould, Prostaglandins *11*, 489 (1976).
3 P.K. Moore, J.R.S. Hoult and A.S. Laurie, Lancet *2*, 98 (1978).
4 P. Sharon, M. Ligumsky, D. Rachmilewitz and U. Zor, Gastroenterology *75*, 638 (1978).
5 E.D. Williams, S.M.M. Karim and M. Sandler, Lancet *1*, 22 (1968).
6 A. Bennett, Nature *231*, 536 (1971).
7 R.K. Farris, E.J. Tapper, D.W. Powell and S.M. Morris, J. clin. Invest. *57*, 916 (1976).
8 J.J. Misiewicz, S.L. Waller, N. Kiley and E.W. Horton, Lancet *1*, 648 (1969).
9 A. Robert, in: Advances in Prostaglandin and Thromboxane Research, p. 507. Ed. B. Samuelsson and R. Paoletti, New York 1976.
10 E. Beubler and H. Juan, Experientia *34*, 386 (1978).
11 G.B. Gerber and K.I. Altman, in Radiation Biochemistry, vol. 2, p. 2. Ed. G.B. Gerber, K.I. Altman and S. Okada. Academic Press, New York and London 1970.
12 S. Lowe and G. Faure, Naunyn-Schmiedebergs Arch. Pharmak. *107*, 271 (1925).
13 J.R. Vane, Nature, New Biol. *231*, 232 (1971).
14 W.G. Unger, I.F. Stamford and A. Bennett, Nature *233*, 336 (1971).
15 J.R. Vane, Br. J. Pharmac. Chemother. *12*, 344 (1957).
16 A. Gilmore and J.R. Vane, Nature *218*, 1135 (1968).
17 V. Eisen and D.I. Walker, Br. J. Pharmac. *62*, 461P (1978).
18 V. Eisen and D.I. Walker, Br. J. Pharmac. *57*, 527 (1976).
19 V. Eisen and D.I. Walker, S.G. Binysh and R.S. Tedder, Agents Actions, suppl. 2, p. 99 (1977).
20 A.T. Mennie, V.M. Dalley, L.C. Dinneen and H.O.J. Collier, Lancet *2*, 942 (1975).

Effects of various media on tissular and cellular structures of the superior cervical ganglion of the rat

M.J. Pébusque, A. Robaglia and R. Seïte[1]

Laboratoire d'Histologie 1, Groupe de Neurocytobiologie, Faculté de Médecine, F-13385 Marseille Cedex 4 (France), *10 January 1979*

Summary. The superior cervical ganglia of the rat have been incubated in vitro for 1 h in basal medium Eagle (BME) with Hanks' salts, BME with Earle's salts, Krebs' solution and NCTC 109 medium. Comparison of the cell areas, established by a semi-automatic quantitative method, shows that the three former induce a 30–35% neuronal retraction, whereas NCTC 109 has no effect. Thus this latter medium seems the best one for studies using incubation of these cells.

In vitro preparations of the superior cervical ganglion (SCG) in different species and especially in the rat are widely used for electrophysiological, biochemical or pharmacological studies dealing with sympathetic nervous system. We have made, for example, studies about metabolism of sympathetic ganglia[2,3], different mechanisms of noradrenaline synthesis[4], synaptic transmission[5] and the functions of adenosine 3′-5′-cyclic-monophosphate (cyclic AMP) and its analogs[6,7]. These in vitro preparations have generally been done with Eagle medium with or without newborn calf serum and bicarbonate buffered Krebs' solution.

A major problem for all in vitro systems is the search for a physical and biochemical medium that optimizes neural cell performances[8]. The purpose of this work is to compare rat's SCG incubated in vitro, for 1 h, in 4 different media: basal medium Eagle (BME) with Earle's salts, BME with Hanks' salts, Krebs' solution, and NCTC 109 medium in order to select the best in vitro system.

Materials and methods. Left and right SCG were removed from 20 Wistar adult rats weighing between 230 and 400 g under Nembutal (40 mg/kg) i.p. anesthesia. The animals were divided into 2 groups. Group 1: in 5 rats, both SCG were dissected out, then treated by immersion fixation without previous treatment; group 2: in 15 rats, both SCG were dissected out and before immersion fixation were maintained into various media for 1 h at 36–36.5 °C (5% CO_2 and 95% O_2).

We have used 4 media: Krebs' solution, BME with Earle's salts, BME with Hanks' salts and NCTC 109 medium. The 3 latter media were used with or without 20% newborn calf serum.

After removal, each ganglion (incubated or not) was fixed in 2.5% glutaraldehyde in 0.177 M monodisodic phosphate buffer (pH 7.35) for 1 h at 4 °C. Then, they were postfixed in a 2% osmium tetroxyde solution (pH 7.4) for 1 h at 4 °C, dehydrated with acetone and embedded in Epon. Each ganglion was cut with a Reichert OMU 3 microtome and sections were contrasted with uranyl acetate and lead citrate and examined in a Siemens (Elmiskop 101) electron microscope. In each group, 1 SCG was taken at random. Semi-thin sections are made and photographied in a contrast microscope.

The pictures (magnification × 560) were used to measure cellular areas; 40 neuron areas were fixed in each group (incubated or not) by a system for semi-automatic quantitative evaluation of images (MOP/AMO 1 Kontron). We have made the calculations of mean area, SEM, SD and variance.

Results. Three morphological criteria were used to compare the efficacy of each solution: general aspect of the ganglia, size and ultrastructural morphology of neurons.

On phase contrast microscopical semi-thin sections: there are great differences between SCG morphology according to media. The most important modification in comparison with the control ganglia, fixed by immersion (figure 1), is a cellular retraction with a very high increase of intercellular spaces and the occurrence of dark neurons: especially observed in SCG incubated in the 2 BME media with newborn calf serum (figures 2 and 3) and in Krebs' solution

Neuronal area of SCG: comparison between SCG control and incubated for 1 h in different media*

	Controls	BME with Hanks' salts + SC	BME with Earle's salts + SC	Krebs' solution	NCTC 109 medium + SC
Area (μm^2) ± 95% SEM (n)	272.16 ± 2.31 (40)	192.19 ± 4.21 (40)	177.84 ± 4.40 (40)	189.27 ± 2.93 (40)	267.19 ± 3.68 (40)
Neuronal retraction (%)		30	35	30	2

SC = 20 per cent newborn calf serum; n = number of samples. * The neuronal area after fixation by perfusion is 318.68 ± 4.19 (40).