both the fasting rat serum as well as the liver perfusate, though statistical significance was not achieved. We have observed an increase HDL cholesterol level in the serum of rats fed ethanol although the values were not statistically different from the controls. This is at variance with the work of Hirayama et al.[21] who found significantly higher levels of HDL in fasting rat serum after chronic alcohol feeding. However, their diet has a considerably higher fat content (35%) compared to ours (5%).

In view of the possible beneficial effects of an increased HDL/LDL ratio towards CHD[18], further studies into the role of alcohol on the metabolism of HDL and LDL are indicated. These will include variables such as duration, frequency and amount of alcohol ingested as well as dietary manipulation (e.g. changing protein or fat content).

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2 G.J. Miller and N.E. Miller, Lancet *1*, 16 (1975).
3 T. Gordon, W.P. Castelli, M.C. Hjortland, W.B. Kannel and T.R. Dawber, Am. J. Med. *62*, 707 (1977).
4 N.E. Miller, D.S. Thelle, O.H. Førde and O.D. Mjøs, Lancet *1*, 965 (1977).
5 G.J. Miller and N.E. Miller, in: High density lipoproteins and atherosclerosis, p.95. Eds A.M. Gotto, Jr, N.E. Miller and M.F. Oliver. Elsevier/North Holland Biomedical Press, Amsterdam 1978.
6 D. Steinberg, Eur. J. clin. Invest. *8*, 107 (1978).
7 E.A. Nikkila, in: High density lipoproteins and atherosclerosis, p.177. Eds A.M. Gotto, Jr, N.E. Miller and M.F. Oliver. Elsevier/North Holland Biomedical Press, Amsterdam 1978.
8 J.J. Barboriak and L.A. Menahan, in: Biochemistry and pharmacology of ethanol, p.587. Eds E. Majchrowicz and E.P. Noble. Plenum Press, New York 1979.
9 A.L. Klatsky, C.D. Friedman and A.B. Siegelaub, Ann. intern. Med. *81*, 294 (1974).
10 K. Yano, G.G. Rhoads and A. Kagan, New Engl. J. Med. *297*, 405 (1977).
11 D. Kozararevic, D. McGee and N. Vojvodic, Lancet *1*, 613 (1980).
12 E.A. Hosein, H. Lee and I. Hofmann, Can. J. Biochem. *58*, 1147 (1980).
13 H. Lee and E.A. Hosein, Life Sci. *29*, 135 (1981).
14 J. Folch, M. Lees and G.H. Sloane Stanley, J. biol. Chem. *226*, 497 (1957).
15 A. Zlatkis, B. Zak and A.J. Boyle, J. Lab. clin. Med. *41*, 486 (1953).
16 P.S. Roheim, D. Rachmilewitz, D. Stein and Y. Stein, Biochim. biophys. Acta *248*, 315 (1971).
17 S.-P. Noel and D. Rubinstein, J. Lipid Res. *15*, 301 (1974).
18 K.I. Baghurst, Med. J. Aust. *2*, 177 (1980).
19 A.F. Lefevre, L.M. DeCarli and C.S. Lieber, J. Lipid Res. *13*, 48 (1972).
20 M.R. Lakshman, A.D. Gupta and R.L. Veech, Lipids *13*, 134 (1978).
21 E. Baraona and C.S. Lieber, J. clin. Invest. *49*, 769 (1970).
22 C. Hirayama, Y. Nosaka, S. Yamada and Y. Yamanishi, Res. Commun. Chem. Path. Pharmac. *26*, 563 (1979).

## Effect of ricin, of its subunits and of modeccin on cAMP level in Yoshida ascites cells[1]

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*Summary.* The B chain of ricin strongly decreases the $PGE_1$-enhanced level of cAMP in Yoshida ascites sarcoma cells, whereas the A chain is ineffective. Modeccin does not have any effect.

Ricin (R-60), from the seeds of *Ricinus communis*[2] and modeccin, from the roots of *Adenia digitata*[3,4] are highly toxic lectins which share the property of inhibiting protein synthesis in vitro. Both toxins consist of 2 unequal subunits[5,12]; the A chain is responsible for the inhibitory effect, and the B chain for cell penetration through specific binding to galactosyl receptors on the cell surface. The 2 toxins differ in haemagglutinating capacity[6,7], are not immunologically closely related, and produce different lesions in rats[9,10]. Nomoto et al.[11] found that ricin lowers the level of 3'-5'-cyclic-adenosine-monophosphate (cAMP) in Yoshida ascites sarcoma cells (YS cells) in vitro, after preincubation with prostaglandin $E_1$ ($PGE_1$), through modification of adenylate cyclase activity, with an increase of the $K_m$ for ATP. This effect of ricin was suppressed in the presence of lactose.

We report here that the isolated B chain of ricin is responsible for the lowering of the cAMP level, whereas the A chain has no effect. Modeccin has no effect on the cAMP level in YS cells.

*Materials and methods.* Ricin (R-60) and its A and B chains were prepared as described by Nicolson et al.[12]. The A chain contamination of the isolated B chain was 1% maximum, as judged from the effect on protein synthesis by a lysate of rabbit reticulocytes. Modeccin was prepared as described previously[5]. $PGE_1$ was a kind gift of the Upjohn Spa (Caponago, Milan), cyclic 8-$^3$H AMP (spec. radioact. 27 Ci/mmole) was purchased from The Radiochemical Centre, Amersham, Bucks. U.K., cAMP and bovine serum albumin from Sigma Chemical Co., St. Louis, Mo, USA, theophilline and activated charcoal from Merck, Darmstadt, West Germany. YS cells were a kind gift of Professor Olivotto, Florence, and were transplanted into anesthetized Wistar rats, weighing 120–150 g. All chemicals used were of analytical grade.

*Determination of cAMP level in vitro.* Before utilization, cells were washed 3 times in NaCl 0.9% and were suspended in Krebs-Henseleit Ringer bicarbonate (KH) medium[13] at a final concentration of $15 \times 10^6$ cells/ml. The reaction mixture contained 5 mM theophilline and cellular suspension in KH medium, and was preincubated for 15 min at 30 °C with shaking. Incubation was started with or without (control) $PGE_1$ (5 µg/ml) at 30 °C with shaking, and after 15 min ricin or modeccin (1 µg/ml) or the separated A and B chains (0.5 µg/ml) were added. At the required times, 1 ml of each sample was centrifuged at 3000 rev/min for

5 min; cells were then resuspended in 1 ml of trichloroacetic acid (TCA) 6% and recentrifuged at the same speed for 15 min. The supernatants were extracted 5 times with 4 vol. of water-saturated ether. cAMP assay was carried out according to the method of Brown et al.[14], binding protein being purified as described by Døskeland et al.[15]. Radioactivity was determined in a Packard Tricarb liquid scintillation spectrometer using an external standard, with a counting efficiency of approximately 33%.

*Results and discussion.* $PGE_1$ enhances the level of cAMP in cultured cells or tissues[16]. In YS cells it rapidly increases the cAMP level to between 2 and 3 times that of the control, a plateau being reached after about 15 min of incubation, which was maintained for at least 60 min thereafter (fig. 1). Ricin (1 µg/ml) without $PGE_1$ did not have any effect on cAMP level (fig. 1). When ricin was added after 15 min of incubation with $PGE_1$, the cAMP level was significantly lowered within 15 min and remained low for at least 60 min thereafter (fig. 2). This effect was obtained, and more markedly, when a molar equivalent of the B chain was used instead of ricin (fig. 2). The effect of the B chain on cAMP was maximum at concentrations between 0.5 and 1 µg/ml. he isolated A chain did not affect the cAMP level, under the same experimental conditions (fig. 2).

No changes in the cAMP level were observed when modeccin was added after 15 min of incubation with $PGE_1$.

The effect of the B chain of ricin on the cAMP level in $PGE_1$-stimulated cells is sufficient to account for the action of the whole toxin. This, and the ineffectiveness of the isolated A chain, strongly suggests that the effect of ricin on the level of cAMP in $PGE_1$-stimulated cells is due to the B chain of the toxin. It is not surprising that the isolated A chain of ricin has no effect on cAMP level, since it is known that this chain cannot bind to and enter into cells[6]. However, the fact that the B chain alone affects cAMP level indicates that the toxic A chain is not required for this effect to occur. It can be assumed that the effect of the B chain is mediated through the same mechanism that operates when the whole toxin is used, i.e. the modification of the adenylate cyclase. Thus, the modification is probably a consequence of the interaction of the B chain with receptors on the cell membrane, and this is consistent with a) the fact that adenylate cyclase is a membrane enzyme[17], b) the greater affinity of the isolated B chain, as compared with whole ricin, for the cell membrane, demonstrated by the stronger haemagglutinating activity of the isolated B chain[6], and c) the rapidity of the effect of ricin or of the B subunit on cAMP, as compared with the effect of the toxin on protein synthesis by whole cells, which occurs after a delay of at least 15 min[18].

Modeccin has no effect on cAMP in vitro and this may be due to different properties of the B chain of this toxin, as compared with the B chain of ricin. It has been observed that modeccin, and hence its B chain, has a much weaker haemagglutinating activity than ricin and its B subunit[7], and that the cell surface receptors for modeccin, as compared to ricin, are different and fewer in number[19].

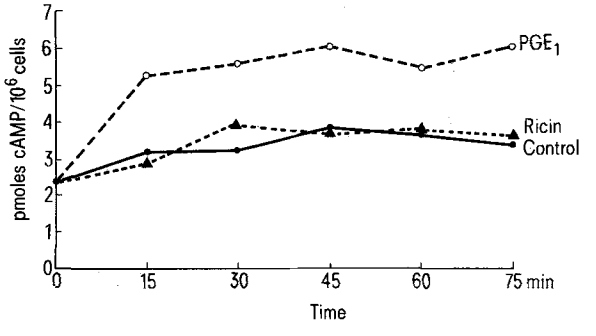


Figure 1. Effect of $PGE_1$ O——O (5 µg/ml) and ricin ▲----▲ (1 µg/ml) on cAMP level in Yoshida ascites sarcoma cells cultured in vitro. Control level is reported ●——●. Experimental conditions are described in the text.

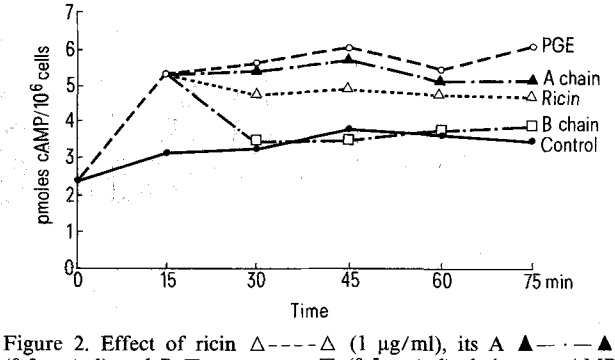


Figure 2. Effect of ricin △----△ (1 µg/ml), its A ▲— · —▲ (0.5 µg/ml) and B □- · - · - · -□ (0.5 µg/ml) chains on cAMP level in Yoshida ascites sarcoma cells cultured in vitro after 15 min of incubation with $PGE_1$ O— —O (5 µg/ml). Control level is reported ●——●. Experimental conditions are described in the text.

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2 S. Olsnes and A. Pihl, in: The specificity and action of animal, bacterial and plant toxins, p. 129. Ed. P. Cuatrecasas. Chapman and Hall, London 1977.
3 K. Refsnes, T. Haylett, K. Sandvig and S. Olsnes, Biochem. biophys. Res. Commun. *79*, 1176 (1977).
4 F. Stirpe, A. Gasperi-Campani, L. Barbieri, E. Lorenzoni, L. Montanaro, S. Sperti and E. Bonetti, FEBS Lett. *85*, 65 (1978).
5 A. Gasperi-Campani, L. Barbieri, E. Lorenzoni, L. Montanaro, S. Sperti, E. Bonetti and F. Stirpe, Biochem. J. *174*, 491 (1978).
6 S. Olsnes and A. Pihl, Biochemistry *12*, 3121 (1973).
7 A. Gasperi-Campani, C. Franceschi, G. Perino and F. Stirpe, Experientia *35*, 1086 (1979).
8 S. Olsnes, T. Haylett and K. Refsnes, J. biol. Chem. *253*, 5069 (1978).
9 M. Derenzini, E. Bonetti, V. Marinozzi and F. Stirpe, Virchows Arch. B Cell Path. *20*, 15 (1976).
10 S. Sperti, L. Montanaro, M. Derenzini, A. Gasperi-Campani and F. Stirpe, Biochem. biophys. Acta *562*, 495 (1979).
11 A. Nomoto, H. Satoh and T. Ukita, J. biol. Chem. *250*, 2703 (1975).
12 G.L. Nicolson, J. Blaustein and M.E. Etzler, Biochemistry *13*, 196 (1974).
13 V. Tomasi, C. Biondi, A. Trevisani, N. Martini and V. Perri, J. Neurochem. *28*, 1297 (1977).
14 B.L. Brown, R.P. Ekins and J.D.M. Albano, Adv. cyclic. nucleot. Res. *2*, 25 (1972).
15 S.O. Døskeland, P.M. Ueland and H.J. Haga, Biochem. J. *161*, 653 (1977).
16 P.W. Ramwell and J.E. Shaw, Rec. Progr. Horm. Res. *26*, 139 (1970).
17 R.R. Bürk, Nature, Lond. *219*, 1272 (1968).
18 S. Olsnes, K. Refsnes and A. Pihl, Nature, Lond. *249*, 627 (1974).
19 S. Olsnes, K. Sandvig, K. Eiklid and A. Pihl, J. supramol. Struct. *9*, 15 (1978).