3',5'-AMP in adipose tissue. These results confirm those of other investigators who found that cyclic 3',5'-AMP was involved in estrogen-induced anabolic effects in rat uterus.[14,15] It must be kept in mind, however, that the adenyl cyclase involved is located in the nucleus whereas the adenyl cyclase promoting lipolysis in adipose tissue is found in the cell membrane.

The present experiments demonstrate a direct estrogen-effect on lipolysis mediated by cyclic AMP. Since, however, the concentration employed in our *in vitro* experiments was fairly high and might be beyond that reached in adipose tissue of the intact animal, further investigations will be necessary to rule out the significance of these mechanisms for estrogen action on lipolysis *in vivo*.

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Salicylate inhibition of *in vitro* plasminogen activation by saline extracts of rat tissues

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MAMMALIAN tissues contain an activator of plasminogen which is readily soluble in saline or potassium thiocyanate solution.[1–2] This activator can promote proteolysis of plasminogen-rich fibrin due to a ready activation of plasminogen to the proteolytic enzyme plasmin. Plasmin action has been shown to be inhibited *in vitro* by sodium salicylate.[3,4] Additionally the antiprotease activity of several non-steroidal anti-inflammatory agents have been reported.[5,6] The present investigation was undertaken to determine the effects of sodium salicylate given intraperitoneally, and acetylsalicylic acid given orally to rats on the proteolysis of plasminogen-rich bovine fibrin plates by saline extracts of various rat tissues.

Male Wistar rats weighing between 150 and 250 g were used in this study. Sodium salicylate (General Biol. Labs) was dissolved in phosphate buffer 0·12M, pH 7·4 and administered intraperitoneally in doses of 50, 100, and 150 mg/kg. Acetylsalicylic acid (BDH) and tragacanth powder in the ratio of 2:1 w/w were mixed with acetate buffer 0·1M, pH 7·4 to make a gummy suspension and administered orally in the same doses (50, 100 and 150 mg/kg). After 90–100 min the rats were sacrificed and brain, heart, kidney, liver, lung and skeletal muscle tissues isolated and collected into ice cold 0·9% w/v sterile sodium chloride solution.

Control rats were given sterile sodium chloride solution intraperitoneally or orally. Ten rats were used as controls for each individual drug and five rats were used for each drug dose. The pooled tissue of each

individual organ was macerated for 10 min in ice cold 0·9% w/v sterile sodium chloride solution in an M.S.E. model macerator at a concentration of 1 g of tissue to 2·5 ml sodium chloride soln. Approximately 2 ml of the macerate was then homogenized for 8–10 min in a Tri-R model homogenizer. The homogenate was diluted to 20 ml with ice cold sodium chloride solution and 0·03 ml aliquots added to bovine fibrin plates.[7] The plates were then incubated in a constant temperature incubator at 37° for 20 hr and lysis zones on the fibrin film recorded as area of lysis in mm^2 by measuring the perpendicular diameter of the zones. This area of lysis was taken as a measure of plasminogen activator activity. The effect of various doses of sodium salicylate and acetylsalicylic acid on the plasminogen activator activity of rat tissue extracts are shown in Table 1. A dose of 50 mg/kg sodium salicylate caused very little change in plasminogen activator activity among various tissues with only brain tissue showing a significant increase ($P < 0·05$) when compared with control. At this same dose acetylsalicylic acid caused significant inhibition in all tissues ($P < 0·005$) except heart and kidney tissues which showed no significance of difference when compared with the control ($P < 0·50$ and $P < 0·80$ respectively). At a dose of 100 mg/kg sodium salicylate, tissue plasminogen activator activity was increased significantly in brain ($P < 0·005$) but reduced in heart, lung and skeletal muscle tissues ($P < 0·01$, $P < 0·005$ and $P < 0·005$ respectively). Acetylsalicylic acid at this dose caused a significant decrease in plasminogen activator activity by all tissues ($P < 0·02$ to $P < 0·005$) with complete inhibition of activator activity by lung tissue. At the high dose of 150 mg/kg sodium salicylate inhibited the degree of plasminogen activator activity by brain, heart and skeletal muscle tissues ($P < 0·01$, $P < 0·005$ and $P < 0·005$ respectively) and completely inhibited lung and kidney tissue activity. Acetylsalicylic acid was more active by completely inhibiting the plasminogen activator activity of all the various tissues except skeletal muscle tissue which caused a measurable but significantly reduced lysis of fibrin ($P < 0·05$). Plasma salicylate levels achieved with sodium salicylate ranged from 11·56 mg% with a dose of 50 mg/kg to 25·15 mg% with a dose of 150 mg/kg. With acetylsalicylic acid, plasma levels ranged from 17·30 mg% with a dose of 50 mg/kg to 31·10 mg% with a dose of 150 mg/kg.

Standard bovine fibrin plates contain significant amounts of plasminogen adsorbed to fibrin but heat treating the plates at 70° for 60 min denatures all adsorbed plasminogen.[8] The presence of a tissue activator of plasminogen is confirmed in this study by the lack of proteolysis with control extracts of tissues on heated bovine fibrin plates. Much of this activator has been reported to be localised in cellular lysosomal fractions.[9] As such it is suggested that the inhibitory effect of salicylates on tissue plasminogen activator activity reported in this study may be due to stabilization of cellular lysosomal membranes preventing the release of activator into tissue extracts. It has previously been demonstrated that acetylsalicylic acid has an *in vitro* stabilizing effect on liver lysosomal membranes.[10] Other anti-inflammatory drugs have

TABLE 1. EFFECT OF SALICYLATES ON THE LYSIS OF PLASMINOGEN-RICH BOVINE FIGRIN PLATES BY SALINE HOMOGENATES OF RAT TISSUES

	Sodium salicylate				Acetylsalicylic acid			
Tissue	Control	50 mg/kg	100 mg/kg	150 mg/kg	Control	50 mg/kg	100 mg/kg	150 mg/kg
Brain	178·068 ± 12·387	220·571 ± 5·838 P < 0·05	251·214 ± 6·030 P < 0·005	106·928 ± 40·195 P < 0·01	222·214 ± 16·009	116·066 ± 3·164 P < 0·005	77·928 ± 22·558 P < 0·02	No lysis
Heart	168·655 ± 12·513	187·333 ± 13·144 P < 0·30	123·642 ± 6·487 P < 0·01	85·466 ± 14·710 P < 0·005	170·785 ± 14·091	184·750 ± 26·489 P < 0·50	59·625 ± 25·745 P < 0·005	No lysis
Kidney	218·689 ± 7·333	240·000 ± 19·329 P < 0·30	257·285 ± 22·179 P < 0·10	No lysis	201·642 ± 8·832	216·857 ± 18·681 P < 0·80	114·500 ± 30·642 P < 0·02	No lysis
Liver	352·035 ± 8·985	355·857 ± 14·911 P < 0·90	360·785 ± 12·851 P < 0·60	148·000 ± 35·735 P < 0·005	321·571 ± 10·238	182·642 ± 29·704 P < 0·005	60·785 ± 11·202 P < 0·005	No lysis
Lung	142·071 ± 6·763	158·857 ± 9·520 P < 0·20	107·812 ± 5·243 P < 0·005	No lysis	169·285 ± 7·238	113·000 ± 3·457 P < 0·005	No lysis	No lysis
Skeletal muscle	321·678 ± 12·949	314·071 ± 14·613 P < 0·80	213·928 ± 15·002 P < 0·005	74·857 ± 27·894 P < 0·005	339·000 ± 14·195	215·357 ± 17·711 P < 0·005	142·142 ± 22·266 P < 0·005	101·785 ± 38·755 P < 0·05

also been shown to have the same effect on liver lysosomal membranes.[11–13] However a recent study has shown that while hydrocortisone has an inhibitory effect on tissue plasminogen activator activity this effect is independent of lysosomal enzymes.[14] It is also possible that the inhibitory effect of salicylates may occur after plasminogen has been activated to plasmin. It is concluded from this study that salicylates are capable of inhibiting rat tissue plasminogen activator activity but the site of action has still to be elucidated.

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Activation by reductive cleavage of potentially cytotoxic azo compounds by human hepatocellular carcinoma

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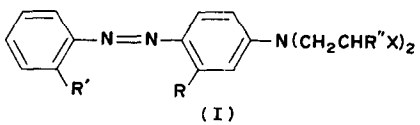
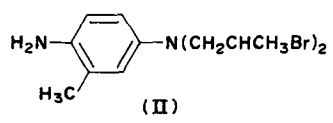
A RECENT report[1] described the synthesis of a series of substituted amino-azobenzene derivatives of the general formula (I) designed to be selective for treating hepatocellular carcinoma. One of these (R = R″ = CH_3, R′ = COOH, X = Br) had the prerequisite properties for selective action; namely absence of alkylating ability and a rapid rate of reduction by rat liver azo-reductase to the amine (II) which is an extremely reactive alkylating agent having a half-life of the same order as the circulation time in man.



(I)



(II)

This compound is undergoing cautious clinical trial in patients suffering from hepatocellular carcinoma in Kenyatta National Hospital, Nairobi, Kenya, and in Kaluva Hospital, Arua, Uganda. Results of these trials will be published elsewhere.