livers. An alternative mechanism would be an oxygen-independent ethanol utilization, in which the NADH produced by ethanol metabolism would be reoxidized by oxidized compounds that could accumulate during the perfusion with DNP. Further work is necessary to fully understand this effect.

Figure 2 shows the results of a group of five experiments done in order to study the effect of DNP in animals fasted overnight, and the reversibility of the effect after a short infusion of the uncoupler. DNP was infused at 50 ± 2 μM. The overnight period of fasting (18 hr, water *ad lib.*) did not alter the baseline metabolism of ethanol when compared to fed animals. This is in agreement with data by other workers[13] showing that, while a 48-hr fasting period markedly reduces the rate of ethanol metabolism *in vivo*, no changes can be observed after a fasting period of 18 hr. DNP increased the rate of alcohol metabolism by about 100 per cent, in agreement with the observations in fed animals and also in line with previous findings in liver slices.[1] The effect of DNP was rapidly reversed under these conditions, in which the liver was exposed for only 5 min to the uncoupler.

In conclusion, the data presented here strongly indicate that the rate of ethanol metabolism is limited by the mitochondrial oxidative capacity.

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Biochemical Pharmacology, Vol. 23, pp. 2337–2339. Pergamon Press, 1974. Printed in Great Britain.

Effect of estradiol on lipolysis and adenosine 3′,5′-monophosphate accumulation in isolated rat adipocytes

(*Received* 10 *October* 1973; *accepted* 31 *January* 1974)

IN RECENT years a considerable amount of evidence has accumulated which demonstrates some influence of estrogens on lipid metabolism. Experiments which were performed mainly after chronic administration of the hormone (14 days and longer) showed an elevation of serum triglycerides[1,2] and total glycerol[3] as well as changes in circulating lipid composition.[3,4] Little is known, however, about the acute effects of estrogens on the metabolism of fat and whether there are any direct estrogen-effects on adipose tissue. In order to get some information on a possible estrogen-induced lipolytic action eventually mediated by cyclic 3′,5′-AMP accumulation we have investigated the influence of estrogens on lipolytic systems *in vivo*

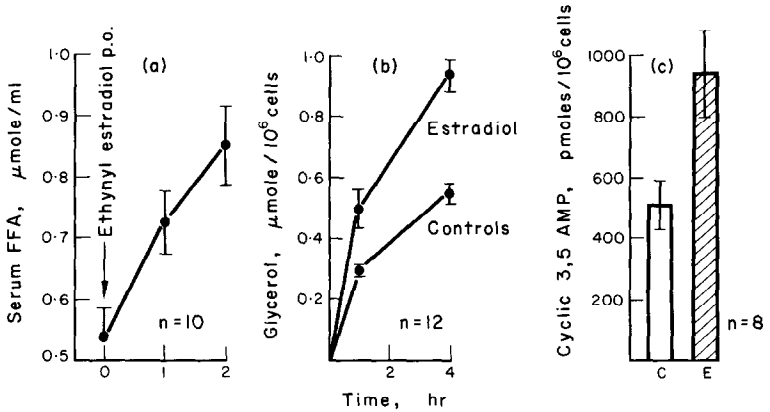

FIG. 1. (a) Effect of 0·1 mg/kg ethynyl estradiol p.o. on serum free fatty acids in white mice. (b) Effect of 10^{-3} M 17β-estradiol on glycerol release in isolated rat epididymal adipocytes (30,000 cells/ml medium). (c) Cyclic 3',5'-AMP accumulation in isolated rat epididymal adipocytes (20,000 cells/ml medium) 5 min after estradiol administration. C = controls; E = 10^{-3} M 17β-estradiol. All values are the mean ± S.E.M. of 8–12 single observations.

and *in vitro*. Serum free fatty acids (FFA) were measured 1 and 2 hr after a single oral dose of ethynyl estradiol, which in contrast to natural estrogens is resorbed perorally to a high degree.[5] Furthermore, the estrogen-effect on glycerol release and cyclic 3',5'-AMP accumulation was investigated in the isolated fat cell.

Female white mice weighing 25 ± 1 g of a laboratory bred NMRI strain which had free access to standard diet (Herilan^R) and tap water were used for the *in vivo* experiments. They received 0·1 mg/kg ethynyl estradiol* in 1·5% methylcellulose soln by intragastric administration and were killed by decapitation after 1 and 2 hr. Blood was collected and serum FFA were estimated according to Duncombe.[6] Animals treated with methylcellulose only served as controls. Lipolytic studies *in vitro* were performed with isolated fat cells from epidydimal adipose tissue of Wistar rats weighing 140–160 g. The fat cells were prepared by a modification of the Rodbell procedure[7] essentially as described by Schwabe *et al.*[8] Aliquots were diluted to 30,000 cells/ml with Krebs–Ringer bicarbonate buffer pH 7·4, containing 2% bovine serum albumin (fraction V obtained from Serva, Heidelberg, dialyzed against buffer), no glucose, and half the recommended concentration of calcium, were incubated in a metabolic shaker at 37° with either 17β-estradiol in 10 μl ethanol to give a final concentration of 10^{-3} M or ethanol alone. Preliminary experiments showed that the ethanol had no effect on lipolysis or the assay techniques. The incubation was terminated after 1 or 4 hr by addition of 0·5 ml 15% trichloracetic acid and glycerol was determined enzymatically according to Eggstein and Kuhlmann.[9] For cyclic 3',5'-AMP determination 20,000 cells/ml were incubated as indicated above for 5–30 min. They were then homogenized in 5% trichloracetic acid in the presence of 0·1 M HCl and cyclic 3',5'-AMP was measured by the protein binding method of Gilman.[10]

As shown in Fig. 1(a), there was a conspicuous rise in serum FFA by 35 per cent after one and by 59 per cent after 2 hr when 0·1 mg/kg ethynyl estradiol was administered p.o. to female white mice. Whereas estradiol (10^{-3}–10^{-6} M) increased lipogenesis in adipose tissue from female rats *in vitro*[11] and had no effect on serum FFA of female rats after long time application[3] our findings indicated that the estrogen was able to stimulate lipolysis in the acute experiment in mice. It was not clear, however, whether this stimulating effect was due to a direct action of the estrogen or was secondary to the release of other hormones involved in the lipolytic mechanism, since estrogens are known to increase the biosynthesis and the secretion of ACTH and somatotropin which in turn may contribute to considerable alterations in lipid metabolism.[3,12]

In order to see whether estrogens had any lipolytic properties by themselves we investigated the influence of the natural estrogen-derivative 17β-estradiol on the lipolysis in isolated adipocytes. It was found that 10^{-3} M estradiol increased lipolysis as measured by glycerol production in rat epididymal adipose tissue cells by 66 and 71 per cent within 1 and 4 hr, respectively [Fig. 1(b)]. As shown in Fig. 1(c), estradiol also increased the cyclic 3',5'-AMP content by 84 per cent after 5 min. The cyclic 3',5'-AMP level was still elevated after 15 min and returned to the control value within 0·5 hr. Such a transient increase in cyclic 3',5'-AMP would be sufficient to produce a significant longlasting stimulation of lipolysis.[13] Therefore it could be assumed that estrogen-induced lipolytic effects were mediated by an accumulation of cyclic

* Kindly supplied by Schering A.G., Berlin.

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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Biochemical Pharmacology, Vol. 23, pp 2339–2341. Pergamon Press, 1974. Printed in Great Britain.

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

(*Received* 12 *November* 1973; *accepted* 12 *February* 1974)

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