Effect of Metopirone and 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)-Pyridine on the Metabolism of Corticosteroids and DMBA in Relation to Adrenal Necrosis

In the adult rat, 7,12-dimethylbenz(a)anthracene (DMBA) causes massive and selective necrosis of the inner zones of the adrenal cortex[1] while the zona glomerulosa and adrenal medulla are spared from damage. The susceptibility of the adrenals could be correlated with their content of corticosterone[2] and it was inferred[3] that this effect was dependent on the functional activity of the adrenal cortex.

Supporting evidence for this theory was provided by CURRIE, HELFENSTEIN and YOUNG[4], who found that adrenal damage by DMBA could be prevented by the concurrent administration of 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (metopirone, Ciba Su-4885) which inhibits 11β-hydroxylation and the synthesis of corticosterone[5,6]. However, 3-(1,2,3,4-tetrahydro-1-oxo-2-naphthyl)-pyridine (Ciba Su-9055), which is a 17α-steroid hydroxylase inhibitor[7], also protects against adrenal necrosis[8] in spite of the fact that the rat adrenal normally does not synthesize 17α-hydroxylated corticosteroids, which suggests that these inhibitors may be preventing the DMBA-induced adrenal necrosis by some other mechanism.

It was therefore considered of interest to investigate the effect of metopirone and Su-9055 administered in vivo on the formation of corticosterone from ^{14}C-progesterone and deoxycorticosterone by rat adrenal homogenates and also on the metabolism of ^{14}C-DMBA by this tissue. The experiments were controlled by adding metopirone or Su-9055 in vitro and by checking their protective action against DMBA under the conditions used to study metabolism, and were repeated twice.

Methods. Young (55 days old) female hooded rats were used and the DMBA (30 mg in 1.5 ml sesame oil) was administered by stomach tube. Metopirone or Su-9055 (15 mg in 0.3 ml oil) was injected i.p. at 09.00 and 12.00 and the animals killed 1 h later or on the following morning (day 2). In both cases the adrenals were removed and incubated with ^{14}C-progesterone or deoxycorticosterone. Another group of rats treated with the hydroxylase inhibitors was given DMBA at 13.00, killed 3 days later and the adrenals examined for gross haemorrhage. Oil alone was injected into the 2 groups of animals serving as control for the adrenal incubations and the test for adrenal necrosis.

Progesterone-4-^{14}C (0.18 μc in 1 μg), deoxycorticosterone-4-^{14}C (0.11 μc in 1 μg) or 7,12-dimethylbenzanthracene-12-^{14}C (0.11 μc in 3 μg) was added to a homogenate of the adrenals (5 mg) in 4 ml 0.1 M potassium phosphate, pH 7.4, and incubated at 38 °C for 1 h under O_2 together with NADP (0.3 mM) and glucose-6-phosphate (3 mM). For the in vitro experiments, inhibitor was added at a concentration of 6.25 μg/ml. The extraction procedure (without acidification) and radioassay were as described previously[9]. The steroid metabolites were examined by paper chromatography in the following systems: toluene saturated with propylene glycol[10], benzene-methanol-water (2:1:1)[11] toluene-methanol-water (4:3:1)[12]. The DMBA metabolites were examined by thin-layer chromatography on silica gel in benzene-ethanol (19:1)[13]. Radioactive products were located by autoradiography and the corticosteroid standards by their UV-absorption or by spraying with 1% tetrazolium blue in 4% methanolic NaOH. Quantitative data were obtained by either cutting out areas on the paper corresponding to darkened areas on the film or by scraping the adsorbent from such areas on the thin-layer sheets into vials and counting directly in scintillation fluid. An aliquot containing 30,000 counts/min was used for chromatography.

Results and discussion. The results indicate that protection from DMBA-induced adrenal necrosis can be obtained with metopirone and Su-9055 under conditions in which there is no apparent change in corticosterone synthesis. No inhibition in 11β- or 21-hydroxylation of progesterone or deoxycorticosterone could be demonstrated in the rat after short-term in vivo treatment with either agent, although metopirone acted as a potent inhibitor in vitro. In addition, no effect on DMBA metabolism was ob-

[1] C. HUGGINS and S. MORII, J. exp. Med. *114*, 741 (1961).
[2] S. MORII and C. HUGGINS, Endocrinology *71*, 972 (1962).
[3] C. HUGGINS and T. SUGIYAMA, Nature *206*, 1310 (1965).
[4] A. R. CURRIE, J. E. HELFENSTEIN and S. YOUNG, Lancet *2*, 1199 (1962).
[5] J. J. CHART and H. SHEPPARD, J. Med. Pharmac. Med. *1*, 407 (1959).
[6] J. ROCHE, R. MICHEL, P. JOUAN and M. CORMIER, Bull. Soc. Chim. biol. *42*, 913 (1960).
[7] H. SHEPPARD and J. J. CHART, Biochem. Pharmac. *8*, 128 (1961).
[8] T. WONG and N. E. WARNER, Endocrinology *74*, 284 (1964).
[9] P. H. JELLINCK and B. GOUDY, Biochem. Pharmac. *16*, 131 (1967).
[10] R. B. BURTON, A. ZAFFARONI and H. KEUTMANN, J. biol. Chem. *188*, 763 (1951).
[11] I. BUSH, Biochem. J. *50*, 370 (1952).
[12] I. BUSH, J. Endocr. *9*, 95 (1953).
[13] E. BOYLAND and P. SIMS, Biochem. J. *95*, 780 (1965).

Effect of metopirone and SU-9055 on the formation of corticosterone and on the metabolism of DMBA by rat adrenal homogenates

Group	No. of rats with adrenal necrosis after treatment with DMBA[a]	Corticosterone formed (counts/min × 10^3)		Metabolites of ^{14}C-DMBA (counts/min × 10^3)	
		^{14}C-progesterone as substrate	^{14}C-deoxycorticosterone as substrate	A(Rf : 0.54)	B(Rf : 0.36)
I Oil control	4/5	11.8 (11.7)[b]	10.7 (12.6)	1.8	0.52
II Su-4885 in vivo	0/5	10.5 (13.1)	10.3 (13.3)	1.8	0.45
III Su-4885 in vitro	–	2.2	2.1	–	–
IV Su-9055 in vivo	0/3	12.4 (13.2)	11.7 (12.1)	1.9	0.50
V Su-9055 in vitro	–	11.2	12.3	–	–

[a] The adrenals from this group were examined for gross haemorrhage but were not incubated with the ^{14}C-steroids or DMBA. [b] Values in brackets are from day 2 of the experiment (see text).

served in animals pretreated with either metopirone or Su-9055.

These findings therefore support the suggestion of WONG and WARNER[8] that interruption of the synthetic pathway of adrenal corticoids is not the only way by which DMBA-induced necrosis can be prevented and that competition between Su-9055 and DMBA at the tissue sites is an important factor. However, we should like to modify this hypothesis, and propose that competition for specific receptor sites in the adrenals occurs between the metabolites of DMBA, metopirone and Su-9055 rather than the original compounds. Thus, there is now strong evidence[14,15] that the proximal necrotic agent is the 7-hydroxymethyl derivative of DMBA, and it is also known[16] that metopirone is converted to a secondary alcohol by rat adrenals. In this respect, it is of interest that Su-9055 also contains a keto group which might therefore be reduced in rat tissues[17].

Résumé. Nous avons protegé des rats contre l'action destructive du DMBA sur les adrénales avec des doses de Su-4885 ou de Su-9055 qui n'avaient pas d'effet sur le métabolisme du progestérone et du déoxycorticostérone. Un mécanisme expliquant l'action de ces substances inhibitrices est suggéré.

P. H. JELLINCK[18], M. GARLAND and D. MCRITCHIE

Cancer Research Centre, University of British Columbia, Vancouver (Canada), 17 October 1967.

[14] E. BOYLAND, P. SIMS and C. HUGGINS, Nature *207*, 816 (1965).
[15] D. N. WHEATLEY, A. G. HAMILTON, A. R. CURRIE, E. BOYLAND and P. SIMS, Nature *211*, 1311 (1966).
[16] M. K. BIRMINGHAM and I. KRAULIS, Proc. 2nd Int. Congr. Hormonal Steroids, Excerpta med. *111*, 251 (1966).
[17] We thank Dr. C. W. MURPHY, Ciba Co. Dorval, for a generous gift of metopirone and Su-9055. This work was supported by the Medical Research Council and the National Cancer Institute of Canada.
[18] Present address: Department of Biochemistry, Queen's University, Kingston, Ontario, Canada.

Ascorbic Acid Oxidizes Thiol Groups of Plasma Proteins

Although ascorbic acid usually functions as a reducing agent, it can oxidize thiols such as glutathione[1]. Oxidation of protein thiol groups by ascorbic acid has not been reported. I have observed oxidation of the thiol groups of human plasma proteins by ascorbic acid in vitro at physiological pH.

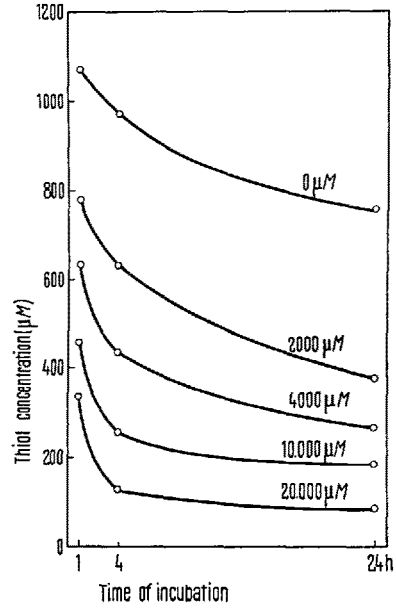
Outdated human blood was obtained from the blood bank, and the plasma was separated by centrifugation. The plasma contained acid citrate dextrose (ACD) anticoagulant.

L-Ascorbic acid was dissolved to make a 0.2 *M* solution in phosphate buffer, and the pH was adjusted to 7.0 with sodium hydroxide solution. An appropriate volume of the ascorbic acid solution was added to 9 ml of plasma, buffer was added to bring the final volume to 10 ml, and the mixture was incubated at 20 °C. Several incubations were done at each concentration of ascorbic acid, with good reproducibility. The same results were obtained when D-araboascorbic acid was used instead of L-ascorbic acid.

Thiol concentrations were measured with 5,5'-dithiobis(2-nitrobenzoic) acid according to the method of ELLMAN[2]. This reagent reacts with both reactive and 'unreactive' thiol groups.

I observed a rapid decrease in the thiol concentration as soon as the ascorbic acid was added. The thiol concentration continued to decrease more slowly for at least 24 h afterwards, as shown in the Figure. This decrease in the thiol concentration is the result of oxidation of thiol groups to disulfide bonds[3]. The rate of oxidation was greater when the concentration of ascorbic acid was increased.

The rapid initial oxidation probably involved the reactive thiol groups, and the slower oxidation that followed probably involved 'unreactive' thiol groups that became accessible to the ascorbic acid as the protein gradually became denatured[3]. A slow oxidation of thiol groups was observed even in the absence of ascorbic acid, as shown by the top curve in the Figure. Because the concentration of glutathione in the plasma is negligible, the major effect of ascorbic acid must be on the protein fraction of the plasma.



Decrease in total thiol concentration of human blood plasma upon incubation with ascorbic acid. The curve labels show the concentrations of ascorbic acid.

[1] P. C. JOCELYN, Biochem. Soc. Symp. *17*, 43 (1959).
[2] G. L. ELLMAN, Archs Biochem. Biophys. *82*, 70 (1959).
[3] R. CECIL, in *The Proteins* (Ed. H. NEURATH; Academic Press, New York-London 1963).