plexes of ferrocysteine (*7*), thus appears to be a distinctive biophysical characteristic of the CO complexes of mixed function oxidases. In addition,

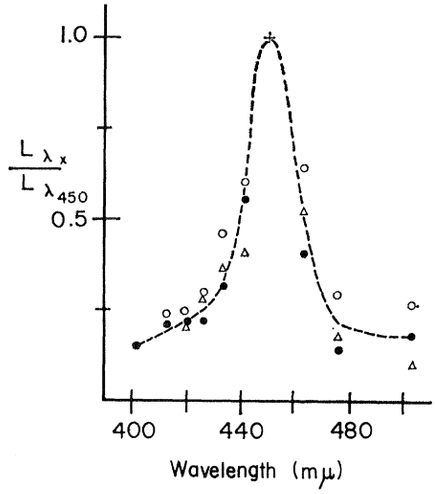


Fig. 3. Photochemical action spectrum of mixed function oxidases of rat liver microsomes. ●, Codeine; ○, MAP; △, acetanilide. Procedure as in Fig. 2. Parahydroxyacetanilide was assayed by a slight modification of the method of Krisch and Staudinger (*16*). Light sensitivity is defined by $L = (1/i)\ (\Delta K/Kd)$, where $i$ is light intensity in terms of mole quanta/(cm² min), $Kd$ the distribution constant in darkness, and $\Delta K$ the increase in $K$ produced by light of a given intensity

$$L_x/L_{450} = (i_{450}/i_x)\ ([\Delta K]_x/[\Delta K]_{450})$$

The numerical values of $Kd$ and of the quantities from which it is derived are recorded in Table 1 which also gives the values of $\Delta K$ and $L$ at 450 mμ.

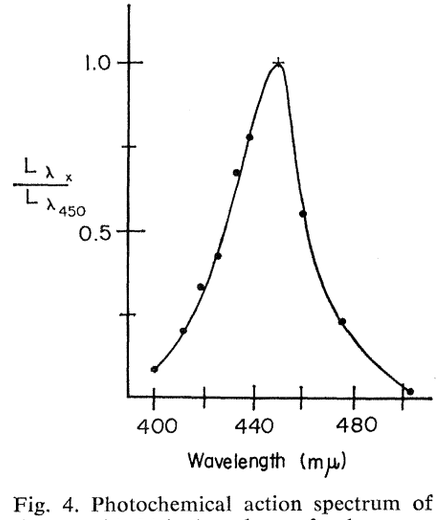


Fig. 4. Photochemical action spectrum of the steroid 21-hydroxylase of adrenocortical microsomes. The procedure has been described (*8*). The microsomal protein was 6.4 mg per vessel; incubation time 15 minutes at 25°C. Numerical data in Table 1.

this property together with the absence of detectable absorption bands outside the Soret region offers an explanation of the failure of Klingenberg (*4*) and Omura and Sato (*6*) to demonstrate spectrophotometrically any dissociation of P-450-CO by irradiating with red light of an intensity sufficient to produce partial dissociation of the CO complexes of either cytochrome $a_3$ or myoglobin.

Besides the accordance between photochemical action spectrum and light absorption difference spectrum of the CO derivatives, the identity of P-450 with the oxygen-activating component of aerobic hydroxylase systems is supported by reports (*11*) that treatment of animals with inducers of aerobic hydroxylase activity of liver microsomes results in a marked increase in microsomal P-450-CO. Conversely we have shown (*12*) that the development of irreversible inhibition after the addition of sulfhydryl reagents to the steroid 21-hydroxylase system is associated with the conversion of the pigment to the irreversibly inactivated derivative defined by Omura and Sato (*6*).

These observations together with our experimental evidence strongly suggest that the so-called carbon monoxide-binding pigment of microsomes is the terminal oxidase of mixed function oxidase systems of mammalian tissues.


DAVID Y. COOPER, SIDNEY LEVIN
SHAKUNTHALA NARASIMHULU
OTTO ROSENTHAL
*Harrison Department of Surgical Research*
RONALD W. ESTABROOK
*Johnson Foundation for Medical Physics, University of Pennsylvania School of Medicine, Philadelphia*


### References and Notes


1. H. S. Mason, *Advan. Enzymol.* **19**, 79 (1957); O. Hayaishi, in *Oxygenases*, O. Hayaishi, Ed. (Academic Press, New York, 1962), p. 1.
2. K. Ryan and L. Engel, *J. Biol. Chem.* **225**, 103 (1957).
3. D. Y. Cooper, R. W. Estabrook, O. Rosenthal. *Federation Proc.* **22**, 530 (1963).
4. M. Klingenberg, *Arch. Biochem.* **75**, 376 (1958).
5. D. Garfinkel, *ibid.*, p. 493.
6. T. Omura and R. Sato, *J. Biol. Chem.* **239**, 2270, 2379 (1964).
7. O. Warburg, *Heavy Metal Prosthetic Groups and Enzyme Action*, (Oxford at the Clarendon Press, 1949).
8. R. W. Estabrook, D. Cooper, O. Rosenthal, *Biochem. Z.* **338**, 741 (1963).
9. H. Remmer and H. J. Merker, *Science* **142**, 1657 (1963).
10. D. Cooper, R. W. Estabrook, O. Rosenthal. *Federation Proc.* **23**, 223 (1964).
11. H. Remmer, presented at Conference on Evaluation and Mechanism of Drug Action, New York Academy of Sciences, 5 March 1964; S. Orrenius and L. Ernster, *Biochem. Biophys. Res. Commun.* **16**, 60 (1964).
12. S. Narasimhulu, D. Y. Cooper, O. Rosenthal. 148th Meeting Am. Chem. Soc., Div. Biol. Chem., Chicago, 1964, Abstr. 27C, No. 41.
13. Jenaer Glasswerke, Schott & Gen.
14. Thin Film Products Corp., Cambridge, Mass.
15. T. Nash, *Biochem. J.* **55**, 416 (1953).
16. K. Krisch and H. J. Staudinger, *Biochem. Z.* **334**, 312 (1961).


17. Supported by an Established Investigatorship of the American Heart Association to Dr. D. Y. Cooper, a USPHS research development award (GM-K-3-4111) to Dr. R. W. Estabrook, USPHS grants AM-07217, AM-04484, RG-9956, and a grant from the Southeastern Pennsylvania Heart Association. The technical assistance of Olga Foroff, Acie Slade, Jeanine Gonze, and Andy Segal, and the collaboration of Dr. M. Schwartz in determining the light intensities are gratefully acknowledged.

19 November 1964


## Cleft Palate in the Mouse: A Teratogenic Index of Glucocorticoid Potency


*Abstract. Clinically equivalent doses of hydrocortisone, prednisolone, and dexamethasone have progressively increasing teratogenic activity as judged by their ability to induce cleft palate in the offspring of pregnant mice treated with these drugs during the middle period of gestation. Mole for mole, dexamethasone is at least 300 times more teratogenic than hydrocortisone. The enhanced teratogenicity of dexamethasone probably does not result from its relatively decreased mineralocorticoid activity.*


A dose of 2.5 mg of cortisone acetate in suspension given intramuscularly to inbred A/Jax mice on each of days 11, 12, 13, and 14 of gestation will produce cleft palate (without cleft lip) in 100 percent of the offspring (*1*). The purposes of our study were: first, to observe the relative teratogenic activity of certain glucocorticoid drugs with cleft palate as the indicator; and second, to compare teratogenicity with other known laboratory parameters of glucocorticoid effect.

Water-soluble preparations of hydrocortisone, prednisolone, and dexamethasone (*2*) were used to avoid variations in preserving and suspending agents. The stability of these solutions was tested by comparing their teratogenic activity when injected within ¼ hour to 4 days of preparation and after 2 weeks of storage at 5°C; no differences were observed. Comparable doses of each steroid were derived from the schedule of therapeutically equivalent dosages used clinically in man (*3*). Since maternal weight is known to affect the frequency of cleft palate after a

standard dose of cortisone acetate (*4*), the treatment groups were selected so that each had a comparable range of maternal weights between 20 and 25 g. The mice were mated overnight; the morning a vaginal plug was observed was taken to be day 0 of gestation. The drugs were administered intramuscularly in the thigh; animals treated on successive days, or with two drugs, were injected in alternate thighs. On day 18 of gestation, the fetuses were removed for external examination.

Cleft palate (without cleft lip) has a spontaneous incidence of 0.3 percent in the A/Jax strain (*5*). It is to be distinguished from cleft lip (with or without cleft palate) which occurs spontaneously in approximately 10 percent of newborn mice of the A/Jax strain. Preliminary trials with the aqueous hydrocortisone solution revealed that injection of 2 mg on each of 4 days produced a very low (3 percent) frequency of cleft palate in the offspring of A/Jax (A) females mated to C57B1 (C) males. The amount of hydrocortisone used subsequently was increased to 4 mg per day, and this was compared with 1 mg of prednisolone and 0.15 mg of dexamethasone. Another group of animals was treated with 0.5 mg of deoxycorticosterone acetate (DOCA) and dexamethasone, simultaneously, in order to assess the effect of a sodium-retaining agent on the process of dexamethasone-induced cleft palate.

The results of treating A/Jax females, bred to C57B1 males, with therapeutically equivalent doses of hydrocortisone or dexamethasone on days 11, 12, 13, and 14 of gestation are presented in Table 1. Dexamethasone was particularly effective in producing cleft palate. The unequal teratogenic effects observed after a single injection of hydrocortisone or dexamethasone on day 13 are compared on lines 7 and 8 (Table 1). These observations indicate a progressive increase in specific teratogenic activity of hydrocortisone, prednisolone, and dexamethasone as the glucocorticoid potency of these steroids increased without commensurate enhancement of their mineralocorticoid effect (*6*). Walker has demonstrated with triamcinolone a similar dissociation between experimental cleft palate activity and relative anti-inflammatory potency (*7*).

With 4 mg of hydrocortisone injected on each of days 11 to 14 of gestation, the increased frequency of cleft palate in the offspring of A/Jax females mated with A/Jax males (18 percent) over that in the offspring of A/Jax females crossed with C57B1 males (3 percent) confirms the influence of genetic factors (*8*) in determining susceptibility to the effect of glucocorticoids.

Table 1. Frequency of cleft palate (without cleft lip) in offspring of pregnant mice treated daily with hydrocortisone (4 mg), prednisolone (0.5 mg), or dexamethasone (0.15 mg) during the middle period of gestation. One group was treated with dexamethasone and deoxycorticosterone (0.5 mg) simultaneously.

| Drug | No. of early and (late) resorptions* | No. of viable embryos† | Frequency of cleft palate (%) |
|---|---|---|---|
| *A/Jax females mated with C57B1 males* | | | |
| Hydrocortisone | 4 | 34 | 3 |
| Dexamethasone | 3 | 41 | 93 |
| *A/Jax females mated with A/Jax males* | | | |
| Hydrocortisone | | 56 | 18 |
| Prednisolone | 11 (1) | 53 | 77‡ |
| Dexamethasone | 4 | 33 | 100‡ |
| Dexamethasone + deoxycorticosterone | 10 (5) | 21 | 100 |
| Hydrocortisone‖ | 6 | 51 | 4 |
| Dexamethasone‖ | 4 | 55 | 55 |

* Differentiated placentae or bits of tissue recognizable as residua of implantation sites are defined as early resorptions. † Excludes those with spontaneous cleft lip and palate. ‡ $p$ value for significance of difference between prednisolone and dexamethasone is less than 0.01. ‖ Drug injected on day 13 of gestation; in all other instances the drugs were injected on days 11 through 14.

Deoxycorticosterone, a potent mineralocorticoid with sodium-retaining properties, does not produce cleft palate in susceptible strains of mice even in doses large enough to kill the pregnant female (*7*). The concurrent administration of deoxycorticosterone and dexamethasone did not reduce the incidence of cleft palate in viable fetuses (Table 1, line 6). Moreover, an unusual proportion of resorbing fetuses with developmental ages characteristic of day 14 to day 16 of gestation were found when the mother was killed on day 18. Since the stage of palate development attained by the older resorbing fetuses was delayed relative to other morphological criteria, it is assumed that these also would have had cleft palates if they had not resorbed. Thus, a dose of deoxycorticosterone, lethal to some fetuses, was unable to protect against the cleft palate–inducing ability of dexamethasone. This suggests that the enhanced ability of dexamethasone to induce cleft palate is not a consequence of its relatively decreased sodium-retaining activity.

Various assays of steroid potency are performed in laboratory animals to provide estimates of clinical efficacy. Measurements of granuloma inhibition, liver glycogen deposition, and thymic involution in the adrenalectomized male rat suggest that dexamethasone is 104, 90, and 47 times, respectively, as potent as hydrocortisone (*9*). By contrast, in man, the eosinopenic and hyperglycemic effects of dexamethasone indicate that it is about 30 times more active than hydrocortisone (*10*), a value which correlates well with its relative clinical anti-inflammatory efficacy. While dexamethasone produces 20 times as much liver glycogen deposition as does hydrocortisone in the mouse (*11*), its "cleft palate activity" is several hundredfold greater than that of hydrocortisone. The data presented here suggest that the comparative activities of dexamethasone and of hydrocortisone as inducers of cleft palate are probably unrelated to their relative glycogen deposition activity.

It is proposed that the pharmacologic characterization of all glucocorticoids should include induction of cleft palate in the mouse as a simple assay of teratogenic activity. Additional studies which correlate teratogenicity both with alterations in molecular structure and with other indices of corticoid activity, may yield clues to the mechanism whereby glucocorticoids interfere with normal closure of the secondary palate in the mouse.

LEONARD PINSKY
ANGELO M. DIGEORGE
*Department of Pediatrics, Temple University School of Medicine, and St. Christopher's Hospital for Children, Philadelphia, Pennsylvania*

**References and Notes**

1. F. C. Fraser, B. E. Walker, D. G. Trasler, *Pediatrics* **19**, 782 (1957).
2. The drugs were in the form of disodium 21-phosphate derivatives; acquired through the courtesy of H. M. Peck, Merck, Sharp & Dohme, West Point, Pa.
3. Doses with clinically equivalent anti-inflammatory activity; cortisone, 25 mg; hydrocortisone, 20 mg; prednisolone, 5 mg; triamcinolone, 4 mg; dexamethasone, 0.75 mg.
4. H. Kalter, *Genetics* **42**, 380 (1957).
5. D. G. Trasler, K. H. Clark, F. C. Fraser, *J. Hered.* **47**, 99 (1956).
6. G. W. Liddle, *Ann. N.Y. Acad. Sci.* **82**, 854 (1959).
7. B. E. Walker, personal communication.
8. H. Kalter, *Genetics* **39**, 185 (1954).
9. L. L. Lerner, A. Bianchi, A. R. Turkeimer, F. M. Singer, A. Borman, *Ann. N.Y. Acad. Sci.* **116**, 1071 (1964).
10. I. Ringler, K. West, W. E. Dulin, E. W. Boland, *Metabolism* **13**, 37 (1964).
11. R. H. Silber, *Ann. N.Y. Acad. Sci.* **82**, 821 (1959).
12. Supported by USPHS research grant AM-06469 (from NIAMD) and by training grant T1-HD-66 (from NICHD). One of us (L.P.) is now at the Department of Human Genetics, University of Michigan Medical School.

21 October 1964