

## **Inhibitory Effect of Chlorpromazine upon the Adrenal Cortical Response to Insulin Hypoglycemia in Man**

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# INHIBITORY EFFECT OF CHLORPROMAZINE UPON THE ADRENAL CORTICAL RESPONSE TO INSULIN HYPOGLYCEMIA IN MAN<sup>1</sup>

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The large number of recent publications concerning the inter-relationships between the central nervous system and the anterior pituitary testifies to the intense interest in this subject (2, 3). Particular attention has been devoted to the effects of hypothalamic stimulation or injury upon pituitary ACTH release and adrenal cortical response. The work of deGroot and Harris (4), Hume and Wittenstein (5), Porter (6), McCann (7), and many others has indicated the importance of hypothalamic integrity in the normal release of adrenocorticotropin from the pituitary in response to a stimulus.

Another approach to the problem of neural control of the pituitary has been made through the use of central nervous system-depressant drugs such as morphine (8), which reportedly exerts an inhibitory influence upon pituitary release of corticotropin in response to acute stressing procedures. Among the drugs so used is the phenothiazine derivative, 10-( $\gamma$ -dimethylaminopropyl)-2-chlorophenothiazine ("largactil," chlorpromazine). A major site of action of this agent has been thought to be the hypothalamus (9). Some weight was lent to this supposition by the finding of Wase, Christensen, and Polley that chlorpromazine labeled with S<sup>35</sup> was accumulated in the hypothalamus in concentrations exceeding those attained in other areas of the brain (10).

Reports of the effect of this drug upon the adrenal response (and by inference, upon the hypothalamic-pituitary-adrenal response) to various

stresses have described conflicting findings. Using rat adrenal ascorbic acid-depletion as the index of adrenal response, Aron (11), Hamburger (12), Ohler, Sevy, and Weiner (13), and Olling and deWied (14) have independently shown that chlorpromazine apparently blocked adrenal ascorbic acid depletion after operative shock. Holzbauer and Vogt (15) found that the drug failed to inhibit this response, and Cheymol, deLeeuw, and Oger (16) were able to show only a partial interference. Additional difficulties arose from the work of Georges and Cahn (17) who observed that chlorpromazine could itself produce eosinopenia in rats; of Egdahl, Richards, and Hume (18) who demonstrated elevations of cortisol levels in adrenal venous blood of intact dogs after intravenous chlorpromazine administration; of Hamburger (12) who showed moderate ascorbic acid depletion after chlorpromazine alone; and of Harwood (19) who reported rises in plasma 17-hydroxycorticosteroid values in monkeys given the drug.

In the face of these conflicting data, the following study was undertaken in an attempt to clarify the effects of chlorpromazine on the adrenocortical response of human subjects to acute stimuli.

## METHODS AND MATERIALS

1. *Experimental procedure.* The stimulus employed in this study was insulin coma as used in the treatment of patients with schizophrenia. The index of pituitary-adrenal response was the rise in plasma 17-OH-corticosteroid levels. Such rises have been reported to occur even after mild insulin hypoglycemia (20). Plasma steroid responses to insulin in schizophrenic patients were shown to be entirely comparable to the normal by Bliss, Migeon, Branch, and Samuels (21).

The procedure was as follows. Nine patients, seven females and two males, aged 15 to 44, who were undergoing insulin coma treatment for schizophrenia were selected. Control blood samples for plasma corticosteroid

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determinations were drawn at 6:30 a.m. Standard insulin was then administered subcutaneously in doses of 45 to 670 units. Two additional blood samples were then drawn, one at the beginning of coma, and the second just before its termination (*i.e.*, at 3 and 4 hours after insulin).

Four to 21 days later the same procedure was repeated after pre-treatment at 4:30 a.m. and at 6:30 a.m. with 50 to 150 mg. of chlorpromazine hydrochloride given orally, for total doses of 100 to 300 mg. This timing was selected in an effort to attain maximal blood levels of the drug at the inception and at the height of coma (9).

Three to 17 days after the chlorpromazine experiment was performed in a given patient, the procedure was repeated without chlorpromazine.

In eight of the nine patients, blood specimens for glucose determination were obtained at 0 time (6:30 a.m.) and 3 hours after insulin administration.

2. *Effect of chlorpromazine upon response of the adrenal cortex to exogenous ACTH.* In order to rule out the possibility that chlorpromazine might exert an effect upon adrenal cortical response to ACTH, patients treated with the drug in large doses (400 to 1,000 mg. per day) for periods of 25 to 70 days were subjected to standardized intravenous ACTH tests in a manner previously described (22), the index of response again being the rise in plasma 17-OH-corticosteroid levels.

In addition, three patients received chlorpromazine acutely, with a dose schedule like that employed in the insulin coma studies, and then were subjected to intravenous ACTH tests.

3. *Analytical methods.* Levels of plasma 17,21-dihydroxy-20-ketosteroids were estimated by the Silber-Porter method (23), as modified in this laboratory (24), and more recently, by a procedure which incorporated the essential features of the changes made by Peterson, Wyngaarden, Guerra, Brodie, and Bunim (25) and which eliminates the need for adding cortisol to the unknowns.

Blood glucose determinations were made according to the method of Benedict (26).

For more specific measurement of plasma 17-OH-corticosteroids, paper chromatography was resorted to. This was deemed necessary because it was noticed as the study progressed that a pink color formed in the blank tubes of plasma samples taken from patients who had received large doses of chlorpromazine. *In vitro* studies revealed that this chromogen was due to chlorpromazine and that it also interfered with optimal development of Porter-Silber chromogen, *i.e.*, with the development of the color reaction between cortisol and phenylhydrazine-sulfuric acid. Readings were reduced, at most, to values 15 per cent below control levels in *in vitro* experiments in which chlorpromazine in amounts equivalent to 250 micrograms per 100 ml. plasma was added to known amounts of cortisol.<sup>4</sup> Attempts to separate chlorpromazine from corti-

sol *in vitro* and *in vivo* by washing extracts with 1 N sulfuric acid (9) and by means of column chromatography (18) were not successful.

Separation was achieved by paper chromatography. Plasma was extracted with ethyl acetate, washed with base, subjected to hexane:methanol partition, and the dried extract chromatographed in a formamide:chloroform system as described by Burton, Zaffaroni, and Keutmann (27). The cortisol region was detected by ultraviolet light absorption and mobility in comparison with a simultaneously chromatographed reference standard of steroid. Quantitation of cortisol (and further confirmation of its probable identity) was carried out after methanol elution from paper by the colorimetric methods of Porter and Silber (28), and (in one instance) Gornall and Macdonald (29). In each case, blanks (sulfuric acid and ethanol without phenylhydrazine or 2,4-dinitrophenylhydrazine) were run on the eluate of the cortisol region of the paper chromatogram. The fact that no pink color was detectable in any of these blanks was taken as evidence that the chlorpromazine had been separated from the steroid. In the 11 samples tested by the Porter-Silber method (28), unknowns formed the usual yellow color with an absorption maximum at 410 millimicra. Corresponding blanks did not give inordinately high readings (4 to 25 per cent of the readings of unknowns) either in the chlorpromazine-treated specimens or in those which were untreated.

Recoveries of known amounts of cortisol ranged from 35 to 75 per cent by this technic. *In vitro* addition of chlorpromazine in amounts as large as 100 micrograms did not alter recoveries of cortisol.

Plasma samples which were subjected to these extraction and chromatographic procedures were obtained from 12 additional patients with insulin coma. Large samples of blood were drawn at 3 and at 4 hours, and the two specimens from a given patient pooled together. Quantitation of post-insulin cortisol levels in the group receiving only insulin was compared with quantitation of the steroid in the group receiving insulin plus chlorpromazine.

The figure, 250 micrograms per 100 ml. plasma, was arrived at after trial and error as a quantity which produced the pink chromogen to a degree greater than that encountered in plasma specimens obtained from the chlorpromazine-treated subjects. Plasma concentrations in the patients were therefore assumed to be less than 250 micrograms per 100 ml. The validity of that assumption appears to be borne out by data presented in a study published since the preparation of this report (Salzman, N. P., and Brodie, B. B., *Physiological disposition and fate of chlorpromazine and a method for its estimation in biological material.* *J. Pharmacol. & Exper. Therap.*, 1956, 118, 46). Dogs receiving 20 mg. per kg. of chlorpromazine intravenously attained plasma levels of 70 to 260 micrograms per 100 ml. during the period, 0.5 to 3 hours after administration. The doses given to the subjects in the present study were of the order of 1.5 to 4.5 mg. per kg.

<sup>4</sup> No reliable quantitative data are yet available concerning the plasma levels of chlorpromazine attained in human subjects given the usual doses of the drug (9).

TABLE I  
Response of plasma 17-OH-corticosteroid levels to  
insulin hypoglycemia  
Initial control experiments

Patient	Insulin (units)	Plasma 17-OH-corticosteroids (micrograms %)		
		Control	3 Hours	4 Hours
J. S.	420	23	37	43
M. K.	390	21	26	39
J. Sc.	500	27	41	53
S. L.	160	19	33	34
R. M.	270	19	48	46
S. R.	50	26	33	45
J. D.	630	20	29	30
I. S.	100	14	27	40
H. M.	320	22	33	44
Average	316	22	34	42

## RESULTS

*Response of plasma 17-OH-corticosteroid levels to insulin coma*

Effects of insulin coma upon plasma 17-OH-corticosteroid values are summarized in Table I. It will be seen that resting levels fell within the normal range (4 to 28 micrograms per 100 ml. [24]), and at 3 and at 4 hours after insulin there was a consistent rise to mean levels of 34 and 42 micrograms per 100 ml., respectively. These levels were above the normal range and were roughly comparable to levels attained by normal individuals following intravenous administration of 25 i.u. ACTH (22). There appeared to be no correlation between the magnitude of plasma 17-OH-corticosteroid rise and the size of the dose of insulin.<sup>5</sup>

*Effect of chlorpromazine upon plasma 17-OH-corticosteroid response to insulin coma*

Table II shows the results in the same patients pre-treated with chlorpromazine. It is apparent that the resting levels of plasma 17-OH-corticosteroids were comparable to those found in the control experiments (mean levels, 20 and 22 micrograms per 100 ml., respectively). In contrast to the control responses, 17-OH-corticosteroid levels during coma did not rise, but remained

<sup>5</sup> This dose was arrived at by purely empirical means, being the dose required to produce satisfactory coma which was not unduly prolonged. The required dose was extremely variable from patient to patient, and tended in most cases to become progressively smaller during the course of the series of coma treatments.

TABLE II  
Effect of chlorpromazine upon plasma 17-OH-corticosteroid  
response to insulin hypoglycemia

Patient	Insulin (mg.)	Chlorpromazine (Total dose)	Plasma 17-OH-corticosteroids (micrograms per 100 ml.)		
			Control	3 Hours	4 Hours
J. S.	400	300	30	23	27
M. K.	410	200	27	10	23
J. Sc.	170	200	23	14	7
S. L.	160	100	27	14	13
R. M.	300	300	22	0	27
S. R.	45	200	15	8	8
J. D.	630	200	11	6	19
I. S.	120	200*	23	15	22
H. M.	300	300	4	8	11
Average	281		20	11	17

\* A dose of 150 mg. chlorpromazine failed to suppress adrenocortical response to insulin hypoglycemia in this patient.

within normal limits. The trend of values was reminiscent of the normal diurnal variation of plasma 17-OH-corticosteroid levels observed by Bliss, Sandberg, Nelson, and Eik-Nes (30). The duration and depth of coma did not appear to differ from the coma seen during the control experiments without chlorpromazine. The only clinical differences noted were diminution of salivation and sweating in the chlorpromazine-treated group. No differences in depression of blood pressure were found.

In the repeat control experiments, plasma 17-OH-corticosteroid levels rose as they had in the initial controls in six of eight subjects (Table III). The data from control and chlorpromazine experiments in four of the patients are presented graphically in Figure 1.

In two additional patients, chlorpromazine did not clearly inhibit the adrenal cortical response to insulin. Since the control rises in plasma 17-OH-corticosteroid levels were also equivocal, it was thought reasonable to exclude the findings in these cases from the data presented.

As shown in Table IV, depressions of blood glucose in the control and chlorpromazine studies did not differ appreciably.

*Chromatographic studies of plasma 17-OH-corticosteroids during insulin coma*

Table V summarizes the results of chromatographic fractionation and quantitation of plasma cortisol levels. In the Table are compared the

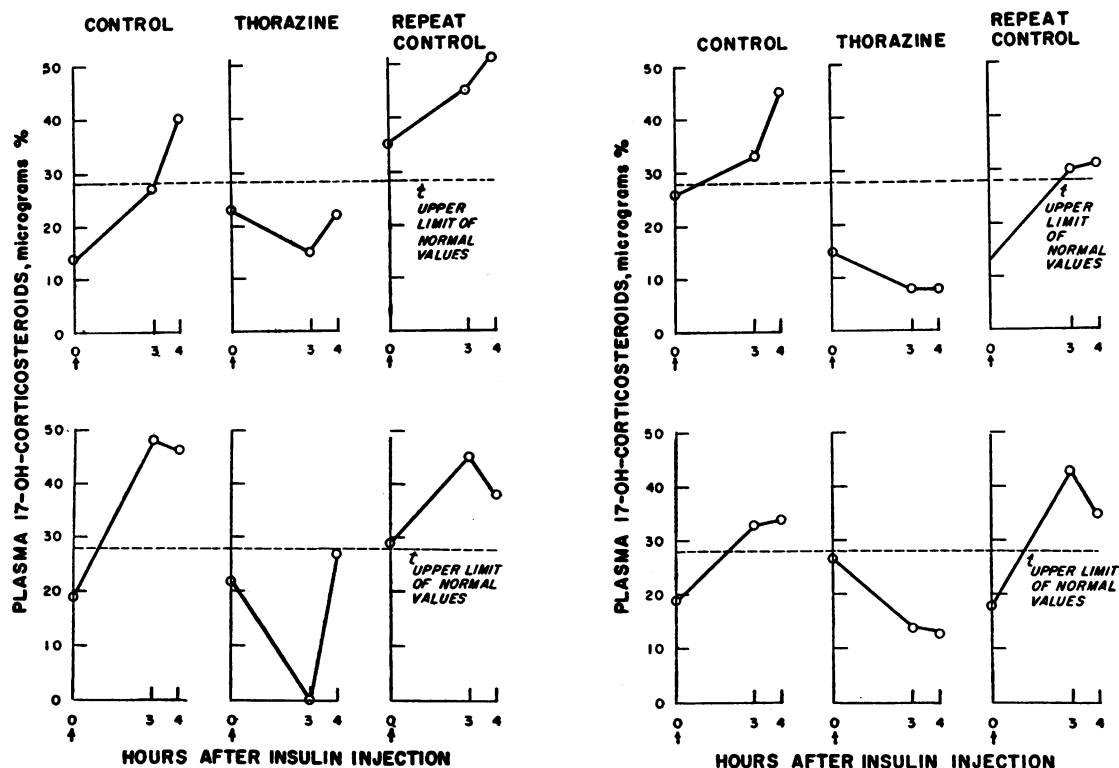


FIG. 1. EFFECT OF CHLORPROMAZINE UPON ADRENOCORTICAL RESPONSE TO INSULIN HYPOGLYCEMIA

Arrows indicate time of insulin injection. Data obtained from patients I. S., S. R., R. M., and S. L. (reading left to right).

amounts of cortisol detected during insulin coma with and without chlorpromazine pre-treatment. Without chlorpromazine, the mean value for plasma cortisol after chromatography was 18.0 micrograms per 100 ml. (without chromatography, 34.6 micrograms per 100 ml.; "recovery," 52 per cent). In the chlorpromazine-treated subjects, the average value was 8.7 micrograms per 100 ml. (without chromatography, 16.7 micrograms per 100 ml.; "recovery," 52 per cent). The difference between the means of the two groups was statistically significant ( $P < 0.01$ ). The fact that about the same proportion of cortisol was recovered in control and chlorpromazine experiments after chromatography lessened the suspicion that the difference in cortisol levels could be accounted for by an adverse effect of chlorpromazine itself upon steroid recovery. This already seemed unlikely from *in vitro* studies (cf. Section 3, *Methods and Materials*).

#### *Effect of chlorpromazine upon plasma 17-OH-corticosteroid response to exogenous ACTH*

Table VI shows the effects of long- and short-term chlorpromazine administration upon the response of the adrenal cortex to exogenous ACTH. It is obvious that only one of the patients (A. T.) showed a very slightly reduced response. Despite the inhibitory effect of the drug upon plasma corticosteroid response to insulin, it did not bring about a suppression of ACTH-responsiveness of the adrenal cortex as does prolonged steroid administration (31). Similarly, acute administration of the drug, given according to the dose schedule used in the insulin studies, had no suppressive effect upon the response to ACTH.

#### DISCUSSION

The data presented confirm the efficacy of insulin hypoglycemia as a stimulus to the adrenal cortex in man (20). The results also indicate

TABLE III

Response of plasma 17-OH-corticosteroid levels to insulin hypoglycemia

Repeat control experiments

Patient	Insulin (units)	Plasma 17-OH-corticosteroids (micrograms %)		
		Control	3 Hours	4 Hours
J. S.				
M. K.	400	37	48	48
J. Sc.*	190	26	27	38
S. L.	160	18	43	35
R. M.	200	29	45	38
S. R.	45	14	30	31
J. D.	670	29	31	34
I. S.	120	35	45	51
H. M.	110	22	17	25
Average	237†	26	36	38

\* This patient received 100 mg. phenobarbital prior to experiment.

† Note that average dosage of insulin is less than in the chlorpromazine experiment (Table II), yet adrenocortical response still occurs in a manner comparable to initial controls (Table I).

that chlorpromazine can inhibit the adrenocortical reaction to this stimulus. The inhibition of plasma 17-OH-corticosteroid rise after insulin which was caused by chlorpromazine does not seem to be an artifact. Chromatographic separation and quantitation of steroid revealed that cortisol in plasma of the chlorpromazine-treated subject was reduced by about 50 per cent in comparison with the control. This was essentially the same degree of reduction found in unchromatographed plasma specimens.

TABLE IV

Lack of influence of chlorpromazine upon blood glucose response to insulin hypoglycemia

Patient*	Blood glucose (mg. per 100 ml.)			
	Without chlorpromazine		With chlorpromazine	
	Control	3 Hours	Control	3 Hours
M. K.			70	30
J. Sc.	97	42	101	39
S. L.			87	29
R. M.	97	30	103	31
S. R.	82	33	94	42
J. D.			103	31
I. S.			69	19
H. M.	94	32	97	34
Average	93	34	91	32

\* In all but one of the patients in whom blood glucose responses were recorded both with and without chlorpromazine, insulin doses were comparable.

TABLE V

Results of chromatographic analysis of cortisol present in plasma of patients during insulin coma, with and without chlorpromazine pre-treatment

Specimen	17-OH-corticosteroids (micrograms per 100 ml.)			
	Without chlorpromazine		With chlorpromazine	
	Before chromatography	After chromatography*	Before chromatography	After chromatography*
1	32.5	21.0		
2		16.4		
3	38.2	12.9		
4	34.9	11.4		
5	36.0	26.0		
6	31.2	20.0		
7			15.2	7.6
8			15.3	9.8
9			20.9	11.2
10			12.3	10.0
11			21.6	8.0
12			15.0	5.8
Average	34.6	18.0†	16.7	8.7†
S.D.		5.5†		1.9†
Cortisol recovered after chromatography (Average, %)		52		52

\* Values after chromatography have not been corrected for losses.

† "T-test" shows statistically significant difference between the mean values (P < 0.01).

The results appear to support those of certain previous investigations in animals (11-14). The absence of definite blockade of adrenal cortical response reported by other workers (15-17) may perhaps be explained by differences in dosage and

TABLE VI

Lack of inhibitory effect of chlorpromazine upon response of plasma 17-OH-corticosteroid levels to a standard intravenous ACTH test

Patient	Chlorpromazine dose/day (mg.)	Duration of treatment (days)	Plasma 17-OH-corticosteroids (micrograms per 100 ml.)	
			Before ACTH	After ACTH
A. E.	400	25	25	59
A. T.	600-1000	30	17	33
H. G.	1000	30	41	63
R. D.	400-1000	70	19	47
J. D.	200	*	12	37
S. R.	200	*	11	37
M. G.	200	*	11	66
Normal range (31)			4-23	35-59

\* Chlorpromazine administered in these three patients according to the dose schedule employed in the insulin coma experiments.

in timing of administration of the drug in relation to the stimulus imposed, as Hamburger has suggested (12). The findings presented here and earlier (11-14) are not necessarily incompatible with the adrenocortical-stimulating property of chlorpromazine demonstrated by Hamburger (12), by Egdahl, Richards, and Hume (18), and by Harwood (19). The factors of speed and route of administration may play a role, and as Harris has pointed out, chlorpromazine may be to some extent a toxic compound like other "blocking agents" which have been used in similar experiments and which themselves cause corticotropin release (2).

This experiment does not elucidate the mechanism of the demonstrated blocking action of chlorpromazine. It seems clear from the findings presented here and from the work of Olling and deWied (14) that the drug does not interfere with the action of adrenocorticotropin upon the adrenal cortex itself. There is no experimental precedent to suggest that this agent can cause an increased rate of disappearance of cortisol from plasma, or accelerated hepatic reduction and conjugation of cortisol. To date, an increased rate of cortisol disposal has been demonstrated only in hyperthyroidism (25).

In trying to visualize the mechanism of the blockade, one is tempted to assume that the site of action of chlorpromazine in inhibiting adrenocortical response (and by inference, pituitary corticotropin release) might be the hypothalamus. Some support for this assumption might be derived from the known suppressive effects of chlorpromazine upon the hypothalamus (9), from the moderately selective accumulation of chlorpromazine in hypothalamic structures (10), and from the knowledge that experimental damage to certain hypothalamic nuclei may result in inhibition of ACTH release in response to a stimulus (4-7). However, such a chain of reasoning must be viewed as entirely speculative, and it should be realized that the data presented in this report, which might be construed as supporting such a concept, constitute only indirect and circumstantial evidence.

The fact that relatively prolonged administration of chlorpromazine failed to suppress plasma 17-OH-corticosteroid response to exogenous ACTH (in contrast to long-term steroid administration [31]) is not necessarily incompatible with

the inhibitory effect of the drug upon adrenal cortical response to an acute stimulus. The two apparently contradictory findings may perhaps be reconciled by Harris' concept of a dual control of the central nervous system over corticotropin release, one mechanism operating under quiescent conditions, the other under conditions of "stress" (2). Such an explanation is again speculative, and it is emphasized that the above discrepancy cannot be satisfactorily accounted for on the basis of present information.

Finally, it is perhaps of some importance that, however produced, blockade of adrenal cortical response to the rather severe stress of insulin hypoglycemia did not result in any clinical symptoms suggesting adrenal cortical insufficiency. Similar observations were made when adrenal cortical response to typhoid vaccine administration was prevented by blocking the pyrogenic reaction with aminopyrine (32). These findings may be interpreted as supporting the concept of the "permissive" role of the adrenal cortex in homeostasis as stated by Ingle (33), and again raise a question as to the advantage for the organism of large increases in adrenal cortical activity in response to acute stimuli.

#### SUMMARY

1. Insulin coma was again shown to cause adrenal cortical response in man, as measured by rises in plasma 17-OH-corticosteroid levels.
2. Chlorpromazine usually inhibited this adrenal cortical response to insulin hypoglycemia.
3. Chlorpromazine had no inhibitory effect upon the response of the human adrenal cortex to administered adrenocorticotropin.

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