CLINICAL EXPERIENCE WITH SELECTIVE INHIBITION OF ADRENAL FUNCTION*

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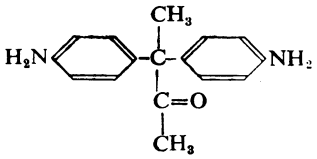
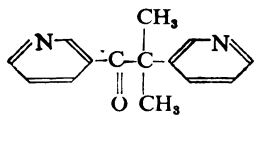
The beneficial effects seen after adrenalectomy in some cases of mammary and prostatic carcinoma and in Cushing's syndrome have stimulated interest in substances capable of inhibiting adrenal cortical function by a direct action on steroid biosynthesis.

The compound which has been studied most extensively is amphenone B (3,3-bis (*p*-aminophenyl)-2-butanone), synthesized by Allen and Corwin (1952). Thorn *et al.* (1956) reported that the levels of urinary and plasma adrenal steroids could be reduced by this drug in man, and a decrease in urinary aldosterone was demonstrated by Renold *et al.* (1957). Thyroid function also was depressed, owing to interference with the



Amphenone B



SU 4885

organic binding of iodine (Hertz *et al.*, 1956). Unfortunately, however, the toxic effects of amphenone have precluded its general clinical use and have led to the search for less toxic inhibitory agents.

Recently, the compound, 2-methyl-1,2 bis-(3-pyridyl)-1-propanone (SU 4885), has been synthesized by Allen and Bencze, and in a preliminary study of this substance Chart *et al.* (1958) obtained evidence of adrenal cortical inhibition in experimental animals. Jenkins *et al.* (1958) demonstrated the inhibitory effect in the dog and showed that, unlike amphenone, SU 4885, at a controlled dose level, could selectively inhibit steroid hydroxylation at position 11. The administration of larger quantities of the drug produced a relatively transient fall in total steroid secretion in the dog; but this was followed by a prolonged period during which hydrocortisone and corticosterone remained suppressed while increasing amounts of 11-deoxyhydrocortisone (Reichstein's compound S) and deoxycortone appeared in the adrenal venous blood. Similar results in man have been reported by Liddle (1958). In view of these findings, the action of SU 4885 on adrenal cortical function in man has been further investigated.

*Supported in part by funds from the United States Public Health Service, Bethesda, Maryland.

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Methods

Eight patients were studied, including four cases of Cushing's syndrome and four who showed no evidence of adrenal abnormality. Five of the patients were given the drug intravenously and the remaining three received it orally.

Urinary 17-hydroxycorticoids were determined by the method of Reddy (1954) and urinary 17-ketosteroids by the method of Drekter *et al.* (1952). Interference with these determinations by urinary metabolites of SU 4885 was discounted by the observation that the administration of the drug to a patient with Addison's disease, who was receiving a constant cortisone dosage, caused no change in the levels of 17-hydroxycorticoids and 17-ketosteroids.

Individual urinary steroids were characterized and estimated by the following technique. A 20-ml. aliquot of urine was incubated with 20,000 units of β-glucuronidase and acetate buffer at *p*H 4.5 for 48 hours at 37° C. The urine was then extracted twice with equal volumes of ethyl acetate and the extract was washed first with normal NaOH, then with water, dried with sodium sulphate, and evaporated to dryness. The dry extract was dissolved in a chloroform-methanol mixture, applied to chromatography paper (Whatman No. 1), and run in the benzene – 50% aqueous methanol system of Bush (1952), together with standard reference steroids. Identification was carried out by a comparison of running rates of the free and acetylated substance with that of the known standards in different chromatographic systems, the blue tetrazolium and phenylhydrazine reactions, and measurement of the absorption spectra in concentrated sulphuric acid. The steroid identified as pregnane-3α,17α,21-triol,20-one (tetrahydro-S) gave, in addition, an infra-red spectrum consistent with this compound. Estimation of pregnane-3α,11β,17α,21-tetrol-20-one (tetrahydrohydrocortisone, T.H.F.), pregnane-3α,17α,21-triol-11,20-dione (tetrahydrocortisone, T.H.E.), and tetrahydro-S (T.H.S.) was carried out by eluting the appropriate areas from the paper with methanol, followed by the application of the phenylhydrazine reaction of Porter and Silber (1950).

Plasma free and conjugated corticosteroids were estimated by a similar technique.

Urinary aldosterone was determined by a modification of the method of Neher and Wettstein (1955) described by Hernando *et al.* (1957).

Effect of Intravenous SU 4885

Table I shows the effect on urinary steroid excretion of an intravenous infusion of 2 g. of SU 4885 given for either four or eight hours. In each case studied there was a large increase in T.H.S., part of which persisted for 12 to 24 hours after the infusion was discontinued. A marked decrease in T.H.F. and T.H.E. excretion was observed in Case 1. In Cases 2 and 3 the decrease was not great enough to be distinguished from possible diurnal variations. Case 4 received the drug over eight hours, and urine collections over 12 hours did not indicate a significant depression of T.H.E. and T.H.F. Case 5 had cirrhosis of the liver and a low four-hour urinary steroid excretion initially, so that a decrease was not easily demonstrable.

Plasma Steroids.—Table II shows the effect on free and conjugated plasma steroids in Case 1. An inhibition of both hydrocortisone (F.) and T.H.F. was

observed with the appearance of 11-deoxyhydrocortisone (S.) and a large quantity of T.H.S. after the infusion of 2 g. of SU 4885 over four hours, even when exogenous corticotrophin was given simultaneously.

Effect of Oral SU 4885

Three patients were given SU 4885 orally in amounts ranging from 3 to 12 g. daily, divided into four-hourly doses.

Total Urinary 17-hydroxycorticoids and 17-ketosteroids (Table III).—In every case the 17-hydroxycorticoids, which include T.H.F., T.H.E., and T.H.S., increased considerably with administration of the drug, and remained elevated above the control values one to two days later. In Cases 6 and 8 urinary 17-ketosteroids were also increased, but in Case 7 no significant rise occurred.

Urinary T.H.F., T.H.E., and T.H.S. (Table IV and Chart).—In cases 6 and 7, who were given only 3 g. initially, urinary T.H.S. rose to a maximum of 20.9 mg. and 11.9 mg. respectively per 24 hours, although no decrease in T.H.E. and T.H.F. was demonstrable. Case 8 was given 6 and 12 g., which resulted in a marked depression of T.H.E. and T.H.F. (see Chart); simultaneously, the output of T.H.S. rose to 28.5 mg.

TABLE I.—*Effect of Intravenous SU 4885 on Urinary Steroids*

Case No.	Age and Sex	Diagnosis	Day	Time	SU 4885	T.H.E. + T.H.F. (mg./4 hr.)	T.H.S. (mg./4 hr.)
1	34 F	Cushing's syndrome	1	3–7 p.m.	—	1·3	0·1
		Adrenal hyperplasia	2	3–7 ,,	2 g. i.v. 3–7 p.m.	0·4	1·5
2	46 M	Cushing's syndrome	1	7–11 a.m.	—	1·8	0
			2	7–11 ,,	2 g. i.v. 7–11 a.m.	1·4	0·4
		Adrenal hyperplasia	2	11 a.m.– 3 p.m.	—	0·8	2·0
			3	3 p.m.– 7 a.m.	—	0·9	0·6
3	42 F	Cushing's syndrome	1	11 a.m.– 3 p.m.	—	3·0	0
			2	11 ,, – 3 ,,	2 g. i.v. 11–3 p.m.	2·6	0·3
		Adrenal adenoma	2	3– 7 ,,	—	1·2	0·7
			3	11 a.m.– 3 ,,	—	2·2	0·5
4	21 M	Cushing's syndrome	1	7 a.m.– 7 ,,	—	4·7*	0·1*
			2	7 ,, – 7 ,,	2 g. i.v. 11–7 p.m.	4·9*	3·8*
		Adrenal hyperplasia	2	7 p.m.– 7 a.m.	—	3·8*	4·0*
			3	7 ,, – 7 ,,	—	4·1*	0·6*
5	57 M	Hepatic cirrhosis	1	3– 7 p.m.	—	0·15	0
			2	3– 7 ,,	2 g. i.v. 11–7 p.m.	0·15	1·1
			2	7–11 ,,	—	0·07	1·0
			3	11 a.m.– 3 ,,	—	0·28	0·4

* mg./12 hours.

TABLE II.—*Effect of Intravenous SU 4885 on Plasma Steroids (Case 1)*

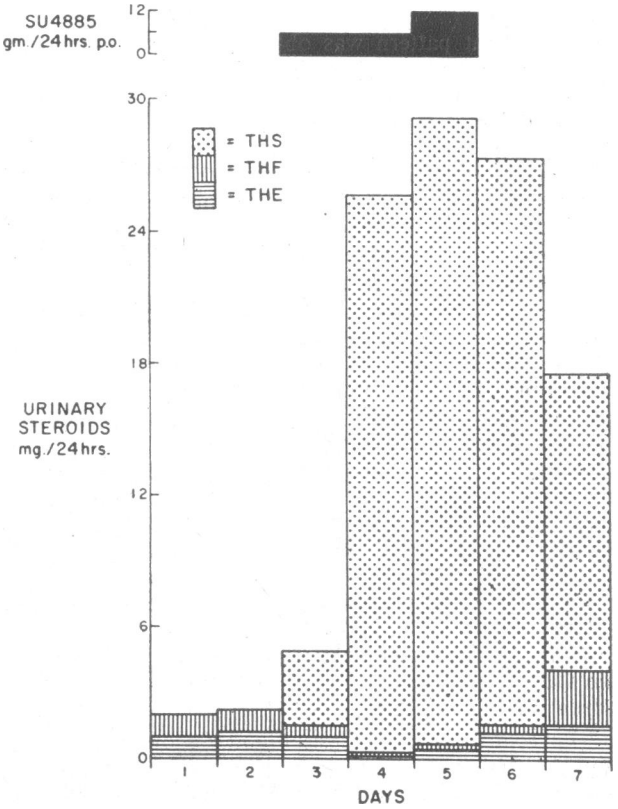
Drug	Time	F. μg./100 ml. Plasma	S. μg./100 ml. Plasma	T.H.F. μg./100 ml. Plasma	T.H.S. μg./100 ml. Plasma
Corticotrophin 25 units i.v. 11 a.m.–7 p.m.	3 p.m.	50	0	20·0	0
SU 4885 .. 2 g. i.v. 3–7 p.m.	7 ,,	15·0	7·0	16·5	56·0

TABLE III.—*Effect of Oral SU 4885 on Total 17-Hydroxycorticoids and 17-Ketosteroids*

Case No.	Age and Sex	Diagnosis	Day	Dose of SU 4885	Total 17-OH (mg./24 hr.)	Total 17 K.S. (mg./24 hr.)
6	62 M	Carcinoma prostate Diabetes Mellitus	1	—	5·5	4·1
			2	—	7·5	3·9
			3	3 g.	8·7	4·2
			4	3 ,,	14·3	8·2
			5	6 ,,	14·0	7·9
			6	6 ,,	24·7	20·5
			7	—	20·9	26·0
			8	—	11·2	16·4
7	71 M	Gout	1	—	5·3	5·7
			2	—	6·2	6·1
			3	3 g.	9·5	5·0
			4	3 ,,	12·4	5·8
			5	6 ,,	12·0	6·6
			6	6 ,,	14·2	5·9
			7	—	15·2	5·9
			8	—	10·0	3·9
8	82 M	Carcinoma prostate Post-castration	1	—	7·7	5·0
			2	—	6·8	5·4
			3	6 g.	11·7	3·2
			4	6 ,,	19·8	10·2
			5	12 ,,	21·5	10·5
			6	—	24·0	8·7
			7	—	7·1	4·2

TABLE IV.—*Effect of Oral SU 4885 on Urinary Steroids*

Case No.	Diagnosis	Day	Dose of SU 4885	T.H.E. + T.H.F. (mg./24 hr.)	T.H.S. (mg./24 hr.)
6	Carcinoma prostate Diabetes Mellitus	1	—	2·8	0
		2	—	4·9	0
		3	3 g.	3·1	0·6
		4	3 ,,	3·0	5·2
		5	6 ,,	2·7	8·5
		6	6 ,,	4·0	20·9
		7	—	3·9	16·5
		8	—	6·6	6·6
7	Gout	1	—	1·6	0
		2	—	1·7	0
		3	3 ,,	1·2	0·3
		4	3 ,,	1·5	2·8
		5	6 ,,	1·5	9·5
		6	6 ,,	0·7	11·9
		7	—	1·7	10·2
		8	—	4·7	5·5
8	Carcinoma prostate Post-castration	1	—	2·1	0
		2	—	2·2	0
		3	6 g.	1·6	3·4
		4	6 ,,	0·2	25·4
		5	12 ,,	0·7	28·5
		6	—	1·6	25·8
		7	—	4·0	7·5



Case 8. Effects of SU 4885 on urinary 17-hydroxycorticoid secretion.

per 24 hours. The elevated levels of 17-hydroxycorticosteroids were therefore due in all cases to the T.H.S. excretion. Two days after stopping the drug T.H.E. and T.H.F. rebounded above the initial control levels, although considerable quantities of T.H.S. were still present.

Sodium, Potassium, and Aldosterone Excretion.—In view of the increased urinary sodium and decreased aldosterone observed during amphenone therapy (Renold *et al.*, 1957) it was of interest to study electrolyte metabolism in the three patients receiving SU 4885 orally while they were receiving a constant dietary intake of sodium and potassium. Table V shows that

TABLE V.—*Effect of SU 4885 on Sodium, Potassium, and Aldosterone Excretion*

Case No.		Day	Dose of SU 4885	Urinary Sodium (mEq/24 hr.)	Urinary Potassium (mEq/24 hr.)	Urinary Aldosterone (μg./24 hr.)
6	Sodium intake 30 mEq/24 hr. Potassium intake 96 mEq/24 hr.	1	—	28	80	
		2	—	23	88	24
		3	3 g.	41	80	16
		4	3 ,,	40	93	28
		5	6 ,,	34	84	20
		6	6 ,,	51	102	26
		7	—	47	79	8
		8	—	26	79	14
7	Sodium intake 10 mEq/24 hr. Potassium intake 90 mEq/24 hr.	1	—	23	96	6
		2	—	12	89	9
		3	3 ,,	14	70	7
		4	3 ,,	24	73	17
		5	6 ,,	10	54	17
		6	6 ,,	5	40	15
		7	—	10	68	5
		8	—	12	54	8
8	Sodium intake 10 mEq/24 hr. Potassium intake 90 mEq/24 hr.	1	—	26	73	
		2	—	23	88	
		3	6 ,,	29	89	
		4	6 ,,	18	71	
		5	12 ,,	11	65	
		6	—	33	77	
		7	—	6	56	

no consistent pattern was obtained. A slight increase in sodium excretion was observed in Case 6, but Cases 7 and 8 tended to retain sodium. Potassium excretion did not change significantly. An attempt was made to measure urinary aldosterone in Cases 6 and 7. No decrease was observed, but results are not easy to assess owing to metabolites of the drug interfering to some extent with the physio-chemical method used for the determination of aldosterone.

A further factor to be considered in the study of electrolyte metabolism during administration of SU 4885 was the tentative identification of pregnane-3α,21-diol-20-one (tetrahydrodeoxycortone, T.H.D.O.C.) a urinary metabolite of deoxycortone (Richardson *et al.*, 1955). A spot was identified on the chromatogram which ran in two different chromatographic systems at the same rate as T.H.D.O.C., gave a positive blue tetrazolium but negative phenylhydrazine reaction, and showed sulphuric acid spectra with maxima at 320 and a plateau from 380 to 400 in accordance with reported values for this compound (Eberlein and Bongiovanni, 1956). Quantitative determination yielded values which rose from initially undetectable levels to a maximum of 0.9 mg. per 24 hours in Case 6 and 0.7 mg. per 24 hours in Case 7, and fell to unmeasurable amounts after the drug was discontinued,

Thyroid Function.—Cases 6 and 7 showed no change in the 24-hour radioactive iodine uptake and serum protein-bound iodine levels, measured before and during the administration of SU 4885.

Carbohydrate Metabolism.—Intravenous glucose-tolerance tests were performed on the three patients before and during the drug period. No significant change was noted. Case 6 was suffering from diabetes mellitus, for which he was receiving tolbutamide, 0.5 g. three times a day. No alteration in dosage was required to control glycosuria while taking SU 4885.

Toxic Effects.—In contrast to amphenone—the chief toxic effects of which are drowsiness, gastric disturbance, methaemoglobinaemia, skin rashes, and possible hepatic dysfunction (Hertz *et al.*, 1956)—reactions due to SU 4885 were much less prominent. No untoward effects were seen in any of the patients receiving an intravenous infusion of the drug. Nausea and gastric discomfort, however, were experienced by all patients taking 6 g. or more orally. This was partly controlled by giving aluminium hydroxide. Case 8 complained of dizziness when he was taking 12 g. daily. Drowsiness was not a feature in any patient. Blood pressure, pulse rate, blood count, urine, and liver function remained unchanged. It should be emphasized, however, that the period of drug administration in no case exceeded four days.

Discussion

A major pathway in the biosynthesis of hydrocortisone involves the formation of 11-deoxyhydrocortisone, which then undergoes 11β-hydroxylation (Hayano *et al.*, 1956). The large quantity of T.H.S. excreted in the urine of all patients treated with SU 4885 demonstrates that the inhibitory action of this drug is directed selectively at the 11-hydroxylase enzyme system. The degree to which hydrocortisone and its urinary metabolites T.H.E. and T.H.F. were suppressed depended upon the dosage administered. When 2 g. was injected intravenously over four hours or 6-12 g. was given orally, a decrease in T.H.E. and T.H.F. could be demonstrated in some cases. With smaller doses inhibition of 11-hydroxylation appeared to be incomplete, and it was postulated that any tendency for the level of hydrocortisone to fall would result in an increased output of endogenous corticotrophin, thereby restoring hydrocortisone to normal, and stimulating further the production of 11-deoxyhydrocortisone. The latter compound has relatively weak biological activity (Bergenstal *et al.*, 1955) and would not itself, therefore, be expected to inhibit corticotrophin secretion. The rise in 17-ketosteroids which was observed in two cases could probably be explained by an increased corticotrophin production.

The effect of SU 4885 on adrenal steroid synthesis is analogous to the congenital failure of 11β-hydroxylation described by Eberlein and Bongiovanni (1956) in some cases of adrenal hyperplasia. These patients excrete large quantities of T.H.S. and also T.H.D.O.C., which is probably responsible for the hypertension seen in this type of congenital adrenal hyperplasia. None of the patients receiving SU 4885 showed any change in blood pressure, but no alteration might be expected during the relatively short period of administration, although T.H.D.O.C. was tentatively identified in the urine of Cases 6 and 7. The inhibitory effect of SU 4885 differs considerably from that of amphenone, which acts by interfering with a number of adrenal enzyme systems (Rosenfeld and Bascom, 1956) with no selective effect on 11-hydroxylation (Jenkins *et al.*, 1959). In the particular dosages used in this investigation SU 4885 did not have the clearly defined effect on the excretion

of sodium and aldosterone possessed by amphenone. Thyroid function also was not affected. SU 4885 is much the less toxic of the two drugs, gastric disturbances being the only consistent symptom. The excessive drowsiness which severely limits the use of amphenone did not occur.

Although the action of SU 4885 on other adrenal steroids, notably oestrogens, has not yet been studied, the relatively large doses required to suppress hydrocortisone together with the formation of 11-deoxyhydrocortisone and probably deoxycortone do not indicate that this particular drug has immediate clinical application as an adrenal inhibitor. On the other hand, it seems likely that further compounds will be elaborated by means of which selective inhibition of adrenal enzyme systems may be satisfactorily achieved in patients.

Summary

The effect of a new adrenal inhibitory compound, SU 4885, has been studied in eight patients. Inhibition is directed particularly at adrenal steroid 11β-hydroxylation, so that 11-deoxyhydrocortisone (compound S) and its urinary metabolite tetrahydro-S were found in relatively large quantities in blood and urine. In sufficient dosage, orally or intravenously, SU 4885 appeared capable of reducing the levels of hydrocortisone and its urinary metabolites.

The toxicity of SU 4885 was significantly less than that of amphenone B.

We are indebted to Drs. Robert Gaunt and C. H. Sullivan, of Ciba Pharmaceutical Products, for the supplies of SU 4885 used in this investigation. Dr. S. Lieberman kindly performed the infra-red spectroscopy. Dr. Nelson appreciates support from the Howard Hughes Medical Institute.

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NORADRENALINE-SECRETING NEUROBLASTOMATA

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Virchow (1864) was probably the first to describe neuroblastoma of the adrenal gland, referring to the tumour as a glioma. Subsequently many such tumours were described as sarcomata. Pepper (1901) regarded his case as one of lymphosarcoma with hepatic metastases. Hutchison (1907) described suprarenal sarcoma in children with metastases in the skull. Wright (1910) established the true nature of the tumour by comparing the rosettes and fibrils with those of embryonic sympathetic nervous tissue. In the light of more recent knowledge the well-known Hutchison and Pepper syndromes are no longer acceptable; Farber (1940) and Karsner (1942) failed to demonstrate any relationship between the site of metastases and the primary tumour, and Bergstrom (1937) failed to show any difference in histology.

The following three cases are pathologically neuroblastomata possessing the peculiar function of catecholamine secretion. On surveying the literature only two other cases are recorded, one by Mason *et al.* (1957) and another possible case referred to by Wilkins (1957).

Case 1

A male child aged 4½ years was admitted to the Isolation Hospital at Boksburg on May 6, 1955, with a history of abdominal pain, headache, and vomiting, which had been recurring at weekly intervals for the preceding three months, increasing in severity and culminating in a fit with left-sided twitching and a short period of unconsciousness.

On examination the child appeared acutely ill. The heart was not clinically enlarged, heart sounds were normal, and the lungs were clear. The liver was enlarged to three finger-breadths below the costal margin, and the tip of the spleen was palpable. The left side of the body was a little weaker than the right. The reflexes were present and equal. There was no neck rigidity. The cranial nerves were intact with the exception of the second, which showed bilateral papill-oedema with numerous exudates. The following day the child experienced a period of blindness lasting 15 minutes; this recurred later in the day and on this occasion lasted four hours. A provisional diagnosis of encephalitis now was made.

On lumbar puncture pressure was normal and the fluid showed no increase in cells or protein. The temperature fluctuated between 98 and 100° F. (36.7 and 37.8° C.). The child received courses of penicillin, streptomycin, and oxytetracycline.

On May 12 the blood pressure was 180/000 mm. Hg; the child was drowsy, and was transferred to the children's ward at Boksburg Hospital for further investigation.

Special Investigations and Progress

Haemoglobin, 15.5 g./100 ml.; erythrocytes, 5,120.000/c.mm.; leucocytes, 15,600/c.mm. (polymorphonuclears