obtained were calculated in μEq/g/h. In order to obtain comparative values, the potency of the peptide was calculated and expressed also in IU.

Results and discussion. The pentacosapeptide described above had an activity of 625.0 IU/mg (F.L. 82.2–121.6%) as estimated by the ascorbic acid depletion assay s.c. This value is remarkable when compared with the values obtained for synthetic β^{1-39} and β^{1-24} corticotrophin. The activities of these peptides and their structural differences are shown in the Figure. This new synthetic peptide is shown to be almost 6 times more potent than β-corticotrophin itself[4] and its β^{1-24} sequence[6].

Estimation of corticosterone release in vitro is used as a direct criterion of adrenal stimulation. Lipolytic activity in vitro is an inherent extraadrenal effect of corticotrophin.

The activity of the pentacosapeptide as determined by these 2 methods is given in the Table.

The value obtained in the corticosterone test was almost twice that obtained in the lipolytic activity test.

Detailed studies of the pentacosapeptide have been made to assess its capacity to stimulate corticosteroid secretion in vivo. Details of these studies will be published separately. It may be briefly mentioned that in these studies, the pentacosapeptide by different routes of administration was again shown to be superior to natural hormone.

ACTH activity in vitro of D-serine[1]-norleucine[4]-valinamide[25]-β-1-25-corticotrophin

Methods	Potency IU/mg peptide	Fiducial limits in % ($P = 0.05$)
Corticosterone release in vitro (SAFFRAN and SCHALLY)[12]	275.0	78.4–127.6
Lipolytic activity in vitro	148.0	80.8–123.7

The outstanding activity of this peptide induced us to test its efficacy in humans. Since the ascorbic acid depletion assay, recommended by most Pharmacopoeias, is generally employed for the standardization of ACTH used therapeutically, it was of great importance to ascertain how far the potency of the new peptide estimated by the ascorbic acid depletion test is related to human dosage.

3 healthy male volunteers received by i.v. injection on alternate days 25 and 50 IU of the new peptide (corresponding to 40 and 80 μg respectively) and the same dosage of a commercial corticotrophin preparation. The subsequent urinary excretion of 17-hydroxycorticosteroids and 17-ketosteroids was estimated and taken as a criterion of the effect. In these preliminary studies, calculating the values as a 4-point assay, the following figures were obtained: 17-hydroxycorticosteroids 840 IU/mg and 17-ketosteroids 580 IU/mg. These figures confirm the high potency of this peptide in humans. A detailed clinical study employing a wider dosage range with frequent blood steroid estimations has been performed by JENNY et al.[15].

The above data clearly demonstrate the remarkable activity of D-serine[1]-norleucine[4]-valinamide[25]-β-1-25-corticotrophin in both animal and human experiments.

Zusammenfassung. Ein ACTH-Analogon: D-Ser[1]-Nle[4]-(Val-NH_2)[25]-β-1-25-Corticotrophin wurde tierexperimentell sowie orientierend humanpharmakologisch untersucht. Das Pentacosapeptid (DW-75) zeigt auf Grund der Substitution von 3 Aminosäuren eine gegenüber Peptiden mit natürlicher Sequenz beachtlich erhöhte ACTH-Aktivität.

W. DOEPFNER

Biological and Medical Research Division, Sandoz Ltd., Basel (Switzerland), May 13, 1966.

[15] M. JENNY, A. F. MULLER, and R. S. MACH, Experientia *22*, 528 (1966).

The Adrenocorticotropic Action of a New Synthetic Pentacosapeptide

Since the description in 1954 by BELL et al.[1,2] of the structural formula of adrenocorticotropic hormone (ACTH), various polypeptides with corticotropic action have been synthesized[3–8]. The polypeptides made up of 19–25 amino acids have an effect comparable to that of an ACTH extracted from pituitaries, are well tolerated and do not appear to exert an antigenic effect[9–16]. BOISSONNAS et al.[17] have recently synthesized a pentacosapeptide with 3 modifications of the usual sequence of naturally occurring ACTH-D-serine[1]-norleucine[4]-valinamide[25]-β-1-25-corticotropin (DW 75). The pharmacological studies of DOEPFNER[18] have shown that this polypeptide has an adrenocorticotropic effect in vitro and in vivo in the rat. This study was undertaken to show that this polypeptide has also an adrenocorticotropic action in man.

Methods. DW 75[19] was given by a single i.v. or i.m. or s.c. injection in doses of 5, 10, 25 and 125 U (corresponding to 8, 16, 40, 200 μg of peptide) at 8 a.m. in patients with no evidence of endocrinopathy or of cardiac, hepatic or renal failure. 4–6 patients were used at each dose level. In order to eliminate the endogenous secretion of ACTH, all patients received 2 mg of dexamethasone orally 8 h before beginning the test, and a further 2 mg were given at the time of the DW 75 injection. Blood was taken for plasma steroid estimation immediately prior to the injection and after 1, 2 and 4 h. The plasma steroids were measured by the method of PETERSON et al.[20].

Results. In 52 control subjects who had received no dexamethasone, the plasma steroid level at 8 a.m. was 15.6 ± 4.6 μg/100 ml. In patients receiving dexamethasone, the level was 2.7 ± 2.2 μg/100 ml at 8 a.m., and this level remained practically constant throughout the day without diurnal variation.

The injections of DW 75, administered i.v., i.m. and s.c., were well tolerated. The changes in steroid levels obtained are shown in the Table and in the Figure. The results are the arithmetic mean with standard deviation of a group of 4–6 patients. The number of patients in each

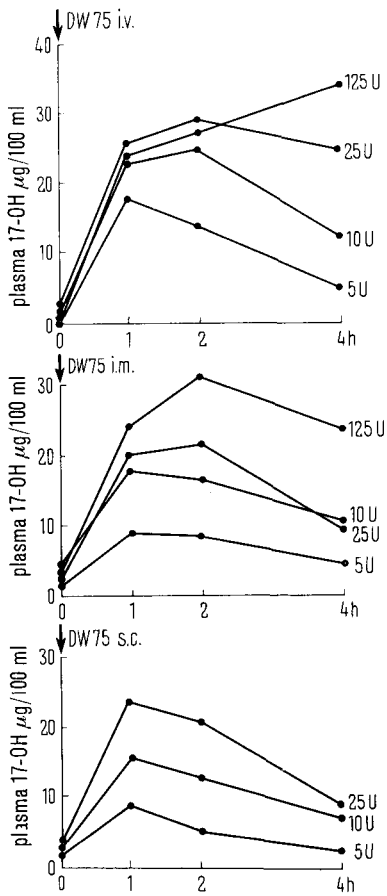
Plasma steroids (μg/100 ml) variations after i.v., i.m. and s.c. injections of 5, 10, 25 and 125 U of a synthetic pentacosapeptide, DW 75. Each result is the arithmetic mean with standard deviation of the plasma steroids of 4–6 patients

			0 h	1 h	2 h	4 h
DW 75	i.v.	125 U	0.7 ± 0.5	23.3 ± 4.9	26.8 ± 2.5	33.7 ± 9.4
	i.v.	25 U	2.2 ± 2.5	25.4 ± 5.3	28.9 ± 6.9	24.9 ± 7.1
	i.v.	10 U	1.6 ± 1.8	22.7 ± 4.1	24.9 ± 11.6	12.4 ± 5.5
	i.v.	5 U	0	17.9 ± 12.7	14.1 ± 7.6	5.1 ± 4.6
DW 75	i.m.	125 U	2.9[a]	24.2	31.3	23.8
	i.m.	25 U	2.5 ± 2.8	20.1 ± 5.3	21.5 ± 4.4	9.5 ± 3.4
	i.m.	10 U	4.2 ± 1.8	17.6 ± 1.3	16.6 ± 4.2	10.5 ± 12.0
	i.m.	5 U	1.3 ± 1.2	9.0 ± 2.5	8.6 ± 3.4	4.8 ± 3.3
DW 75	s.c.	25 U	2.9 ± 1.9	24.1 ± 5.1	21.3 ± 3.5	9.0 ± 4.3
	s.c.	10 U	2.7 ± 3.4	16.0 ± 5.0	12.9 ± 6.0	7.6 ± 4.8
	s.c.	5 U	2.1 ± 3.5	9.4 ± 5.5	5.4 ± 4.8	2.6 ± 2.2

[a] This plasma level is the arithmetic mean of only 2 patients.

group is too small to permit statistical analysis of the results.

5 U of DW 75 by each of the 3 routes of administration cause a distinct rise in plasma steroid level after 1 h. Increase of the injected dose of peptide produced a further increase in steroid levels, reaching a maximum 2 h after 10 and 25 U i.v. and 4 h after 125 U. With a dose of 125 U, the steroid levels began to decrease after 4 h, but were nevertheless 19.5 μg/100 ml after 6 h and 12.5 μg/100 ml after 8 h. The increase in steroid levels was more marked after i.v. than after i.m. or s.c. injection.



Plasma steroid variations after injections of 125, 25, 10 and 5 U of an adrenocorticotropic synthetic polypeptide, DW 75.

Discussion. D-serine[1]-norleucine[4]-valinamide[25]-β-1-25-corticotropin is a synthetic pentacosapeptide possessing, when injected i.v., i.m. or s.c., a potent adrenocorticotropic action in man. By intravenous administration a greater and longer-lasting effect was apparent compared to i.m. and s.c. injections (Figure). One could assume that the polypeptide is more rapidly inactivated in muscular and subcutaneous tissues, and therefore the blood level of adrenocorticotropic substance is lower than with the same dose administered i.v.

[1] P. H. Bell, J. Am. chem. Soc. *76*, 5565 (1954).
[2] R. G. Sheperd, S. D. Willson, K. S. Howard, P. H. Bell, D. S. Davies, S. B. Davis, E. A. Eigner, and N. E. Shakespeare, J. Am. chem. Soc. *78*, 5066 (1956).
[3] R. A. Boissonnas, St. Guttmann, J. P. Waller, and P. A. Jaquenoud, Experientia *12*, 466 (1956).
[4] St. Guttmann, J. Pless, and R. A. Boissonnas, Acta chim. hung. *44*, 141 (1965).
[5] K. Hofmann, H. Yajima, M. Yanaihara, T. Y. Liu, and S. Lande, J. Am. chem. Soc. *83*, 487 (1961).
[6] H. Kappeler and R. Schwyzer, Helv. chim. Acta *44*, 1136 (1961).
[7] C. H. Li, J. Meienhofer, E. Schnabel, and J. Ramachandran, J. Am. chem. Soc. *82*, 5760 (1960).
[8] R. Schwyzer, W. Rittel, H. Kappeler, and B. Iselin, Angew. Chem. *72*, 915 (1960).
[9] A. E. Axelrod, A. C. Trakatellis, and K. Hofmann, Nature *197*, 146 (1963).
[10] T. S. Danowski, K. Hofmann, H. Yajima, and C. Moses, Metabolism *10*, 835 (1961).
[11] P. H. Forsham, V. C. Diraimondo, E. G. Biglieri, and C. H. Li, Metabolism *10*, 335 (1961).
[12] R. Jaques, All. appl. Immunology *28*, 221 (1965).
[13] M. Jenny, A. F. Muller, and R. S. Mach, Schweiz. med. Wschr. *93*, 766 (1963).
[14] E. Maeder and M. Schwarz-Speck, Dermatologia *129*, 59 (1964).
[15] W. Schuler, B. Schaer, and P. Desaulles, Schweiz. med. Wschr. *93*, 1027 (1963).
[16] A. Villanueva, S. J. H. Ashcroft, and J. P. Felber, Acta Endocr. *51*, 88 (1966).
[17] R. A. Boissonnas, St. Guttmann, and J. Pless, Experientia *22*, 526 (1966).
[18] W. Doepfner, Experientia *22*, 527 (1966).
[19] Kindly supplied by Sandoz Ltd., Basel, Switzerland.
[20] R. E. Peterson, A. Karrer, and S. L. Guerra, Anal. Chem. *29*, 14 (1957).

Increasing the injected dose does not result in a significant rise in the maximal steroid levels, but prolongs the duration of adrenocortical stimulation. A dose of 5 U appears to stimulate the adrenals for only 1 h. Doubling the dose results in 2 h stimulation, while with 125 U, or 200 μg of peptide, the effect lasts about 4 h.

In equivalent dosage, DW 75 causes more prolonged adrenal stimulation than a polypeptide containing only 24 amino acids of the natural ACTH sequence[21]. This more prolonged duration of action seems to result from the delayed enzymatic breakdown of the synthetic polypeptide suggested by in vitro studies[17]. DW 75 is the first synthetic polypeptide with an adrenocorticotropic action which has, due to its slower enzymatic breakdown, an effect apparently more prolonged than naturally occurring ACTH[22].

Résumé. L'action adrénocorticotrope d'un polypeptide à 25 acides aminés, comprenant 3 modifications de structure par rapport à la séquence 1–25 de l'ACTH naturelle est étudiée et démontrée. La destruction enzymatique de ce pentacosapeptide paraît être plus lente dans le sang que dans les tissus musculaire et sous-cutané. L'augmentation de la dose injectée produit un allongement de la durée de stimulation de la cortico-surrénale.

M. JENNY, A. F. MULLER, and R. S. MACH

Department of Medicine of the University of Geneva, Hôpital cantonal, Geneva (Switzerland), May 13, 1966.

[21] M. JENNY, A. F. MULLER, and R. S. MACH, Commun. Soc. suisse méd. interne, Lausanne, 15 mai 1966.

[22] The technical assistance of Miss S. WÖRNER and Miss M. NAGTEGAAL is gratefully acknowledged.

Tetracycline Toxicity

We have shown previously that tetracycline induces tubular necrosis in the kidneys of rats if hemoglobinuria is present and have suggested that this might be due to either a short period of ischaemia produced by the tetracycline or to a direct nephrotoxic effect of tetracycline[1]. In either case the effect of tetracycline would be potentiated by the presence of hemoglobin[2,3]. In the present paper the toxicity of tetracycline is examined further and in particular experiments are described which were carried out in order to examine the possibility that tetracycline might induce a transient ischaemia in the kidney.

A total of 90 female albino rats of the Wistar strain were used. Tetracycline was given by intravenous injection into the tail vein in a dose of 15 mg/100 g body weight.

In the first series of experiments 3 different methods were used in an attempt to demonstrate ischaemia in the kidney.

In some rats the abdomen was opened and the appearance of the kidney following the injection of tetracycline observed directly in normal light. This has been found to be a satisfactory method of detecting transient periods of ischaemia in the kidney[2]. There was no change in the appearance of the kidney during the 15 min following the injection.

In other rats 2 ml of Indian ink was injected rapidly into the abdominal aorta at various times up to 15 min after the injection of tetracycline. The kidneys filled normally with Indian ink on all occasions and there was no evidence of patchy ischaemia.

In a further group of rats the rate of exogenous creatinine clearance was measured during the period immediately following the injection of tetracycline and in control animals which received a similar volume of isotonic saline. The method used was that described by MUNTWYLER and GRIFFIN[4], and the tetracycline was given immediately before the collection period. The results of this experiment are given in the Table. They do not show any evidence of a reduction in glomerular filtration rate during the 30 min following the injection of tetracycline.

The above experiments failed to demonstrate any significant ischaemia in the kidneys following the administration of tetracycline. This finding has some importance experimentally as well as clinically in view of the fact that tetracycline has been used to assess renal blood flow[5,6].

In the next series of experiments an attempt was made to see if any toxic effect of tetracycline would be manifested in the presence of other nephrotoxic agents.

Rats were given tetracycline as before and then immediately after the injection either the renal pedicle was clamped for periods varying between 15 and 60 min or one of the following nephrotoxins was given by subcutaneous injection in the amounts indicated per 100 g body weight; potassium dichromate 6 mg, D-L serine 75 mg, uranium nitrate 30 mg. Control animals were treated in exactly the same way except that they were not given tetracycline. All the animals were killed 24 h later and histological sections prepared from the kidneys. The extent of the tubular necrosis following each procedure was

Substance injected before collection period	No. of animals	Body weight (g)	Creatinine clearance (ml/min/100 g body weight)
Tetracycline	10	214	0.65 ± 0.15[a]
Isotonic saline	10	215	0.70 ± 0.19[a]

[a] Standard deviation.

[1] E. TAPP and M. B. LOWE, Br. med. J. *i*, 143 (1966).
[2] R. CARROLL, K. KOVÁCS, and E. TAPP, J. Path. Bact. *89*, 573 (1965).
[3] M. B. LOWE, J. Path. Bact., in press.
[4] E. MUNTWYLER and G. E. GRIFFIN, Am. J. Physiol. *173*, 145 (1953).
[5] E. TAPP, R. CARROLL, and K. KOVÁCS, Experientia *20*, 393 (1964).
[6] K. KOVÁCS, R. CARROLL, and E. TAPP, Archs Path. *78*, 442 (1964).