flavonoids studied showed no detectable variations, whether or not they were in the presence of serum proteins.

The degree of binding ($v$) was defined by the equation:

$$v = \frac{S_P}{S_T} = \frac{E_{int.} - E_{ext.}}{E_{int.}} = 1 \frac{E_{ext.}}{E_{int.}}$$

$S_P$: The quantity of protein-bound flavonoid. This quantity is proportional to the difference in the extinction of the solution inside ($E_{int.}$) and outside ($E_{ext.}$) of the dialysis sack. $S_T$: The total quantity of the flavonoid. This quantity is proportional to $E_{int.}$.

*Results.* Figure 2 shows the percentage of protein-bound molecules in proportion to concentration. Within the range of the concentrations studied (0.1 to 0.5 mg/ml), it was found that several *O*-$\beta$-hydroxyethyl derivatives of rutin (mono-7-HR and tri-7,3',4'-HR) undergo a reduced binding percentage as the concentration increases.

The Table shows the percentage of protein-bound molecules for a concentration of 0.1 mg/ml of the various flavonoids studied. We observe that in the case of non-substituted rutin the binding percentage is very high – 70.9%. As substitutions increase, the percentage progressively diminishes, down to 5.4% in the case of complete substitution of all phenol groups – tetra-5,7,3',4'-HR.

When the protein-rutin complex or the protein-*O*-$\beta$-hydroxyethyl-rutin complex is dialyzed against the 0.1 *M* tri-phosphate buffer at pH 7.4, the bond is destroyed and the flavonoid is quantitatively recovered.

*Discussion.* As we see in the Table, the binding percentage diminishes progressively with the substitution of phenolic OH groups by hydroxyethyl groups. The minimum binding is obtained with the completely substituted derivative – tetra-5,7,3',4'-HR – 5.4% at a concentration of 0.1 mg/ml. The binding of rutin to serum proteins is therefore obviously due to the phenolic groups, whereas the OH groups of the sugars and the ketone group of the heterocyclic nucleus play a very small role in the binding process.

The orthodiphenol grouping at 3',4' plays an important role in the serum protein bond. The substitution at 4', which reduces the orthodiphenol group to a monophenol, produces a great reduction in the binding percentage – from 52.5% for the mono-7-HR to 21.6% for the di-7,4'-HR. The percentage diminishes only by 10% when the phenolic OH is also substituted in position 3', as in the case of tri-7,3',4'-HR. The various bondings are completely reversible.

Percent of binding (± standard deviations) of rutin and its hydroxyethyl derivatives to serum proteins at concentrations of 0.1 mg/ml

| Substances tested | Degree of binding (% ± SD) at a concentration of 0.1 mg/ml | No. of experiments |
|---|---|---|
| Rutin | 70.9 ± 1.4 | 11 |
| Mono-7-HR | 52.5 ± 2.7 | 11 |
| Di-7,4'-HR | 21.6 ± 2.7 | 9 |
| Tri-7,3',4'-HR | 11.6 ± 1.9 | 10 |
| Tetra-5,7,3',4'-HR | 5.4 ± 3.7 | 11 |

All binding percentages are significant against a null linkage. In the paired-test, $p$, in all cases is less than 0.005, except for tetra-5,7,3'4'-HR: $p < 0.05$.

## Depressor Effect of Synthetic Peptides Related to ACTH on Blood Pressure in Rats

M. NAKAMURA, S. MATSUDA, M. UEDA and A. TANAKA

*Shionogi Research Laboratory, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 (Japan), 19 September 1975.*

*Summary.* The depressor effects of natural and synthetic ACTH peptides were demonstrated in the rat. This is an extra-adrenal action of ACTH and is not related to the adrenal-stimulating or melanocyte-stimulating activity of the peptide.

The depressor effects of natural ACTH and an analog, [Gly[1]]-ACTH(1-18)$NH_2$, have been demonstrated in rabbits and cats[1]. The present study offers further evidence that the depressor effect is an extra-adrenal action of ACTH and is not directly related to the adrenal-stimulating or melanocyte-stimulating activity of the peptide.

Peptides were dissolved in 0.005 *N* HCl-0.9% NaCl solution and administered into the tail vein of urethane (1.2 g/kg body wt, s.c.)-anesthetized male Wistar rats weighing 140–160 g at a volume of 0.1 ml per 100 g body wt. The systolic blood pressure was measured by the tail-cuff method[2] using a Physiograph Desk Model DMP-4B with programmed Electro-Sphygmomanometer PE-300 (Narco Bio-System Inc., Houston, Texas). The carotid blood pressure was measured directly by connecting a polyethylene tube (PE 50) inserted into the artery with an electric manometer (MP-4T, Nihon Khoden Kogyo, Tokyo).

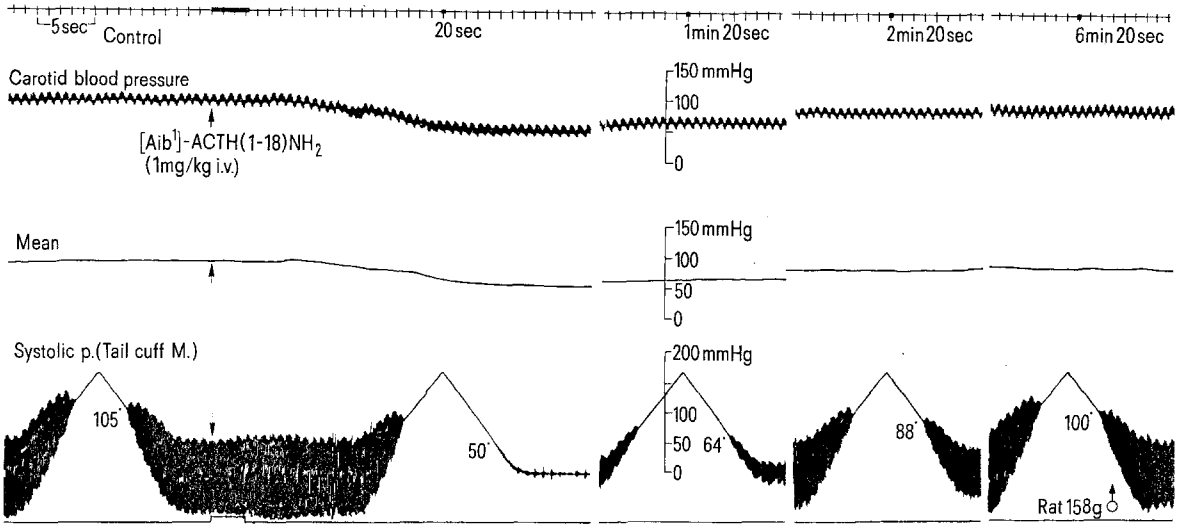
Administration of porcine ACTH (0.5 mg/kg) into the adrenalectomized rat decreased the blood pressure by 40–50 mm Hg with a 2–3 min lag and the reduced pressure was maintained for more than 6 min. [Aib[1]]-ACTH(1-18)$NH_2$(I)[3] at doses of 0.125, 0.25 and 0.50 mg/kg produced immediate decreases of 40–50 mm Hg in the tail blood pressure of adrenalectomized rats and the reduced pressures were maintained for 1, 2 and more than 5 min, respectively.

In intact male rat, a typical response to I is shown in the Figure. The depressor responses with 1 mg/kg of I were 55 mm Hg in the tail (bottom section of the Figure) and 40 mm Hg in the carotid artery (top section). Administration of control vehicle, [Aib[1]]-ACTH(1-10)OH(2 mg/kg), and ACTH(11-18)$NH_2$(2 mg/kg) into the intact rats showed no effect on either the blood pressure or the pulsation in the tail. Synthetic $\alpha$-MSH and human $\beta$-MSH at a dose of 0.25 mg/kg had no effect on intact

[1] M. UEDA, S. MATSUDA, M. KAWAKAMI and H. TAKEDA, Jap. J. Pharmac. *20*, 585 (1970).

[2] R. D. BUNAG and E. RILEY, J. appl. Physiol. *36*, 621 (1974).

[3] Aib: $\alpha$-aminoisobutyric acid.




Effect of [Aib$^1$]-ACTH(1-18)$NH_2$[3] on arterial pressure in rat.

rats. [$\beta$-Ala$^1$, D-Phe$^7$, Orn$^{15}$]-ACTH(1-18)$NH_2$(II) at a dose of 0.25 mg/kg decreased the tail blood pressure by 40–50 mm Hg in intact rats and its potency was almost equal to that of I. On the whole, I, II, and synthetic porcine ACTH seemed to have a similar depressor potency.

Previous experiment in this laboratory have shown that the relative MSH potencies against human $\beta$-MSH in vivo of I, II and synthetic porcine ACTH were 1, 100 and 0.2 on weight basis respectively[4,5] and their ACTH potencies were 480, 6 and 180 units/mg, respectively[6,7]. These results suggest that the depressor activity of ACTH peptides may not be correlated with their melanocyte-stimulating and adrenal-stimulating activity.

Since the depressor response to ACTH seemed to vary from species to species[1], further studies regarding this are now in progress.

[4] M. Nakamura, A. Tanaka, M. Hirata and S. Inoue, Endocr. jap. *19*, 383 (1972).
[5] M. Nakamura and A. Tanaka, Endocr. jap. *19*, 395 (1972).
[6] A. Tanaka, Endocr. jap. *18*, 155 (1971).
[7] M. Nakamura, J. Biochem. *71*, 1029 (1972).

## The Accumulation Pattern of Ingested Gossypol in Selected Organs of the Rat

J. N. Tone and D. R. Jensen

*Department of Biological Sciences, Illinois State University, Normal (Illinois 61761, USA), 11 September 1975.*

*Summary.* Bound gossypol levels in the spleen and kidney of rats ingesting dietary gossypol (0.98%) varied directly with the feeding intervals of 6, 14, 28 and 35 days. Free gossypol level in the kidney, spleen and lungs increased for 14 days and then tended to decrease as the feeding period was extended.

Dietary gossypol accumulates in various organ tissues of swine[1]. Studies have shown that the gossypol concentration in swine tissue is directly related to the level of free gossypol consumed and to the duration of the feeding period, up to 28 days[2]. The administration of a single dose of $^{14}C$-labeled gossypol to rats resulted in an initial high concentration of $^{14}C$ in those organs most likely involved in the elimination of gossypol[3]. However, $^{14}C$-radioactivity in the spleen, lungs, and kidneys was undetected by 13 days post-injection. The liver retained a high radioactivity. The relationship of the duration of the gossypol feeding period to gossypol accumulation in organ tissues of the rat have not been reported. This investigation was designed to determine the distribution of gossypol and its pattern of accumulation in selected organs of rats ingesting gossypol for feeding periods of varying duration.

*Methods.* Male rats (56 days of age) of the Holtzman strain were housed individually in metabolism cages under uniform conditions of light (10 h light, 14 h dark) and temperature (68–72 F). Animals were randomly divided into 2 diet groups. The compositions of the control and experimental diets are shown in Table I. The animals in each diet group were also randomly subdivided into 4 groups of 9 animals, each subgroup corresponding to feeding intervals of 6, 14, 28, and 35 days. The liver, kidneys, spleen, and lungs were collected at the end of

[1] F. H. Smith, Am. Oil Chem. Soc. *42*, 145 (1965).
[2] M. P. Sharma, F. H. Smith and A. J. Clawson, J. Nutr. *88*, 434 (1966).
[3] M. B. Abou-Donia, C. M. Lyman and J. W. Dieckert, Lipids *5*, 938 (1969).