between the compounds with a nitrogen or a sulfur atom in the heterocyclic ring must be responsible for the large differences in their biological activity. The conformational possibilities for the saturated 6-ring include the twist, the boat, and the chair forms. Cyclic imines (piperidine derivatives) are known to assume mostly the chair conformation like cyclohexane derivatives. The introduction of sulfur into the 6-ring might be expected to cause conformational deviations as compared to cyclohexane and piperidine derivatives, e.g., changes in the relative chair, boat, and twist energies or deviations from the perfect chair. These deviations can arise in the case of cyclic sulfide derivatives because of the greater C–S bond length (= 1.82 Å, C–N = 1.47 Å, C–C = 1.52 Å), the smaller C–S–C bond angle (= 100°, C–C–C = 111.5°, C–N–C = 112.6°), and other factors relating to the geometry of the ring system. However, the cyclic sulphonium compounds III and VI appear to exist in a conformation which can more easily react with the muscarinic receptor than the piperidine analogues.

The Binding of Polyphenols (Rutin and Some of its *O*-β-Hydroxyethyl Derivatives) to Human Serum Proteins

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Summary. A binding of polyphenols (rutin and its *o*-β-hydroxyethyl derivatives) to human serum was demonstrated. The results showed an increase of binding proportional to the number of free phenolic groups on the molecule of rutin.

Rutin or rutoside (Figure 1) is a flavonoid with 4 phenolic groups which may be substituted to different extents by *O*-β-hydroxyethyl groups. Rutin and its *O*-β-hydroxyethyl derivatives are therefore especially well suited as a model for a comparative study of protein binding as function of the number of free phenolic groups.

In fact, many studies have demonstrated that the degree of binding of the active principles of drugs to proteins strongly affects their physiological activity. A strong bond, for example, reduces the initial concentration of the free active principle in the blood and at the same time prolongs the duration of its activity[2–6].

In therapy, rutin, and particularly its *O*-β-hydroxyethyl derivatives (HR), exert a protective effect on capillary fragility[7].

The results in this paper show a decrease of binding proportional to the number of substituents.

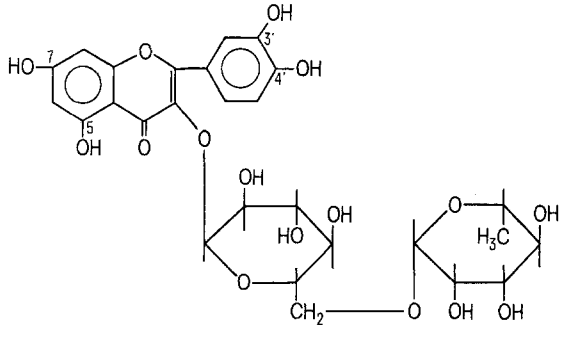

Fig. 1. Rutin.

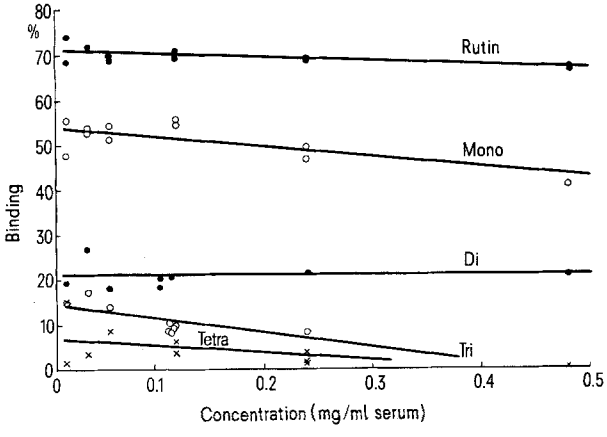

Fig. 2. Degree of binding (%) of rutin and its hydroxyethyl derivatives to human serum proteins, in relation to their concentration. Values determined by equilibrium dialysis.

Materials and methods. Human serum was provided by the Centre for Blood Transfusion in Berne. The serum was dialyzed against an 0.1 triphosphate buffer at pH 7.4 and filtered through an 0.45 μm millipore membrane. Rutin and the *O*-β-hydroxyethyl rutosides (HR) were supplied by the chemical research service of Zyma SA, Nyon. These consisted of mono-7-HR, di-7,4′-HR, tri-7,3′,4′-HR, tetra-5,7,3′,4′-HR[8]. The dialysis membranes were supplied by Kalle SA, of Wiesbaden, West Germany.

The method used was equilibrium dialysis[9]. The dialysis sacks contained 10 ml of serum, in which the product to be tested was dissolved in concentrations from 0.1 to 2 mg/ml. The recipients surrounding the sacks contained 20 ml of the 0.1 *M* triphosphate buffer at pH 7.4. The sacks were agitated at 4 °C until the point of equilibrium was reached after 3 days. Concentrations of the flavonoids inside the dialysis sacks were determined by measurement of the extinction at 350 nm (345 nm for tetra-5,7,3′,4′-HR and 360 nm for rutin). The tests carried out showed that the absorption spectra of the

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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*-β-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*-β-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

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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 α-MSH and human β-MSH at a dose of 0.25 mg/kg had no effect on intact

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