16 times higher concentration of dimethylselenide compared to the exhalation after administration of the same selenium species in the drinking water.

Even after 22 days of selenium administration via drinking water the amount of volatile selenide metabolites is still increasing. On the 14th day after starting the administration less than 0.3% of the daily intake of selenium is exhaled as volatile selenides. These findings are in contrast with similar experiments carried out on rats with selenite, where up to 22% is excreted via the lungs[4,5]. Sternberg and Imbach[6] confirmed the importance of the excretion by lungs for administered selenite as found by McConnell and Roth[5], but they were unable to detect any pulmonary excretion of selenium in rats administered D,L-selenomethionine.

Once more this proves that the chemical form of the added selenium is important in the bioconversion of this element. The identification of the 3rd selenium species in the breath of mice after administration of selenomethionine, and the underlying mechanisms of conversion, remain to the elucidated.

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Tryptophan and neutral amino acid concentrations in serum of rats after salmon calcitonin injection

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Summary. After a single injection of salmon calcitonin (2 MRC units/kg b.wt) marked decreases in both calcium and neutral amino acids in rat serum were observed. In turn, free tryptophan in serum and serotonin (5-hydroxytryptamine) in the whole brain were greatly enhanced during the initial period.

Although the level of plasma and brain free tryptophan has been reported to vary in the same direction under various experimental conditions[2,3], Nakhla et al.[4] have observed that in the rat, after a single injection of calcitonin, the level of plasma free tryptophan diminishes while the level of serotonin in brain increases. This peculiarity could be a consequence of the action of calcitonin on neutral amino acids, which share with tryptophan the same active system for crossing the blood-brain barrier[5]. This could be the reason why prior administration of valine could inhibit the increase of brain tryptophan under the influence of various stresses[6]. To investigate this, we have studied the influence of calcitonin on the levels of various neutral serum amino acids and on the level of brain serotonin in the rat.

Material and methods. All experiments were carried out at 09.00 h using Sprague Dawley male rats weighing 100 g, from 2 different breedings, all fed for at least 8 days with 2 standard laboratory chows. The 2 different laboratory chows were provided by 'Usine d'alimentation rationnelle (régime AO4), Villemoisson (France)' and 'Etablissements Piètremont (régime M.25), Sainte-Colombe, Provins (France)'. The animals were fasted for 16 h before the experimentation but had free access to drinking water. Salmon synthetic calcitonin (Sandoz), diluted in 0.1 ml of a gelatin suspension was administered at a level of 0.5–1 or 2 MRC units per 100 g weight of rat. In order to observe any nonspecific effect of the hormone, some rats were injected with a performic acid oxidation inactivated calcitonin[7], or with a M-sulfoxide calcitonin, and to determine whether the effect was due to reduced calcium concentration, the blood level of calcium in other animals were lowered with an EDTA injection (6×10^{-4} moles/kg)[8]. Control animals received the same amount of gelatin suspension. The animals were sacrificed by decapitation 45 min, 90 min or 4 h after the administration of the hormone. Blood was collected in test tubes without anti-

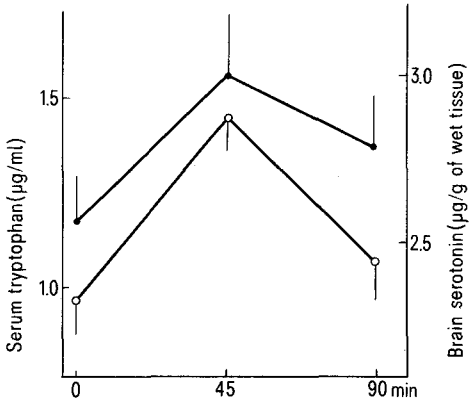

Figure 1. Variations of the serum free tryptophan (○), and brain serotonin (●) concentrations after a calcitonin injection (2 MRC U/100 g, b.wt).

coagulant (heparin, like other anticoagulants, can modify the tryptophan-serum albumin binding) and centrifuged at 4 °C at 2000 × g for 30 min 1 h after collection. The serum samples were kept at −30 °C prior to amino acid analysis. We have verified that freezing under these conditions does not modify the ratio of free tryptophan to bound tryptophan. Brains were immediately removed after decapitation and frozen in a cryostat at −30 °C. Total serum was determined by atomic absorption spectrophotometry (Unicam SP 90). The concentration of tryptophan was determined by the method of Denkla[9], modified by Lehman[10] and Bloxam[11] using a Jobin et Yvon spectrofluorometer. The free fraction of tryptophan was obtained by ultrafiltration of serum using Amicon CF 50 membranes and centrifugation at 500 × g[12]. Total tryptophan was determined following protein precipitation using 10% TCA and centrifugation at 2000 × g for 40 min[13]. The other amino acids were separated and quantitatively determined using the ninhydrin reaction following the method of Stein and Moore[14], after ion exchange chromatography. The concentration of brain serotonin, 5-hydroxytryptamine was determined by a semi-automatic method described by Curzon[15]. The results of each experiment were statistically analyzed and compared using Student's t-test.

Results. The mean concentration of serum calcium in control animals was 9.9 ± 0.2 mg/100 ml. Injection of denatured or M-sulfoxide calcitonin did not modify this value. The administration of 0.5; 1; or 2 MRC units of calcitonin gave a hypocalcemia of 8.0 ± 0.2; 8.2 ± 0.2; 7.9 ± 0.2 mg/100 ml respectively after 45 min and 9.8 ± 0.3; 7.4 ± 0.3; 6.5 ± 0.4 mg/100 ml respectively after 4 h. The administration of 6×10^{-4} moles/kg of EDTA gave a level of calcium of 7.2 ± 0.5 after 45 min and of 9.7 ± 0.6 mg/100 ml after 4 h. The concentration of total tryptophan in serum was 11 ± 1 µg/ml in fasted and 15 ± 2 µg/ml in fed animals. We found that the values did not vary in animals of either breeding, nor did the 2 chows have any influence on them. The administration of sCT had no influence on the total (i.e. free and bound) serum tryptophan, but increased free serum tryptophan; 1.5 ± 0.1 µg/ml in calcitonin treated animals compared to 1 ± 0.1 µg/ml in control animals. This increase disappeared 90 min later. The brain serotonin concentration was 2.5 ± 0.2 µg/g of wet tissue weight in control animals and increased significantly (about 45%) after the administration of 2 MRC units of sCT. The increase was 3.5 ± 0.3 µg/g and 3 ± 0.25 µg/g after 45 and 90 min respectively (fig. 1). The variations of serum concentrations of amino acids 45 min after 2 MRC units of sCT injection are shown in table 1. With the exception of methionine, a significant decrease in the concentrations of neutral amino acids (valine, leucine, isoleucine, tyrosine, $p < 0.01$) was observed (fig. 2). Moreover, it must be mentioned that a decrease of some other serum amino acids was also observed but was not statistically significant (table 1). 45 min after EDTA administration the blood level of neutral amino acids was slightly lower (5%) than controls.

Table 1. The effect of i. p. administration of 0.5–1-2 U MRC of salmon synthetic calcitonin on the concentration of serum amino acids after 45 min

	Control (6 rats)	0.5 U CT/100 g (4 rats)	1 U CT/100 g (4 rats)	2 U CT/100 g (6 rats)
Taurine	217 ± 115	175 ± 30	166 ± 26	223 ± 80
Aspartic acid	47 ± 8	35 ± 3	35 ± 5	38 ± 3
Threonine	280 ± 40	280 ± 20	226 ± 34	249 ± 30
Serine	386 ± 61	347 ± 15	327 ± 34	340 ± 47
Glutamic acid	364 ± 60	309 ± 15	349 ± 42	283 ± 22
Glutamine*	803 ± 454	724 ± 315	551 ± 208	858 ± 211
Proline	138 ± 37	119 ± 10	121 ± 19	116 ± 40
Glycine	703 ± 78	553 ± 42	554 ± 40	550 ± 70
Alanine	497 ± 61	419 ± 81	503 ± 70	482 ± 94
Valine	239 ± 26	214 ± 22	167 ± 24	163 ± 27
Methionine	49 ± 10	56 ± 6	55 ± 7	59 ± 6
Isoleucine	152 ± 13	127 ± 12	99 ± 11	97 ± 32
Leucine	226 ± 28	182 ± 23	143 ± 19	145 ± 37
Tyrosine	81 ± 5	76 ± 9	61 ± 7	66 ± 8
Phenylalanine	96 ± 17	94 ± 3	78 ± 7	80 ± 16

Values are the mean ± SD of 4–6 animals and are expressed in µm/l. The concentrations of the neutral amino acids decrease significantly ($p < 0.01$) after the administration of the hormone. The sensitivity to the degree of oxidation of glutamine seems to be the reason for the non-consistency of the results*.

Table 2. Serum amino acid concentrations of rats injected, 45 min before, with equivalent of 2 MRC units of denaturated synthetic salmon or M-sulfoxide calcitonin or 6×10^{-4} moles/kg EDTA

	Control (6 rats)	EDTA (5 rats)	Denaturated M-sulfocalcitonin
Taurine	242 ± 16	258 ± 13	247 ± 12
Aspartic acid	83 ± 6	79 ± 3	85 ± 6
Threonine	458 ± 55	399 ± 5	442 ± 21
Serine	577 ± 20	483 ± 40	593 ± 31
Glutamic acid	662 ± 64	640 ± 106	558 ± 77
Glutamine	671 ± 111	480 ± 63	636 ± 92
Glycine	853 ± 59	781 ± 76	837 ± 21
Alanine	602 ± 93	562 ± 81	575 ± 38
Valine	261 ± 4	241 ± 17	233 ± 10
Methionine	52 ± 5	50 ± 3	51 ± 5
Isoleucine	168 ± 15	152 ± 17	161 ± 13
Leucine	218 ± 10	200 ± 31	223 ± 16
Tyrosine	80 ± 14	75 ± 9	74 ± 5
Phenylalanine	111 ± 11	103 ± 15	115 ± 11

Values are mean ± SD 5–6 animals, and are expressed in µm/l. The injection of EDTA diminishes the concentration of neutral amino acids by 5% approximately.

The administration of denatured or M-sulfoxide calcitonin did not produce any variation of the concentration of these amino acids (table 2).

Discussion. So far, to our knowledge, the influence of calcitonin on serum neutral amino acids has never been reported. With the exception of the concentration of methionine, which is increased, the concentrations of the neutral amino acids decrease. At present we have no explanation for the different behavior of methionine; it has been shown that the administration of this amino acid to a parathyroidectomized rat results in hypocalcemia[16]. However, it is possible that our observation with methionine is the superposition of various effects. Moreover, the determination of the other amino acids enabled us to show a slight but general decrease of their concentration; this is probably due to a nonspecific stress effect, as has already been shown in the case of an administration of 0.9% NaCl solution[17]. Hypocalcemia could also contribute to the decrease of the concentration of neutral amino acids. But 45 min after the administration of any of the 3 doses of calcitonin the same level of hypocalcemia was obtained, while the neutral amino acid concentrations were different. Moreover the administration of EDTA results in a 25% hypocalcemia and only 5% variation of amino acid concentrations. Thus it seems that a direct effect of calcitonin could be considered.

In mammals, tryptophan is exclusively provided by the food[18]. However, environmental factors during the birth of newborn rats influence tryptophan metabolism[19,20]. For these reasons, various authors state that the mean value of plasma tryptophan concentration varies from 10 to 20 µg/ml[21,22]. As mentioned above, the 2 breedings of the animals, as well as the 2 chows used in our experiments, had no influence on the values of the tryptophan level in the serum. In our experiments, fasting for 16 h results in a decrease of total tryptophan concentration. This decrease has been observed by different authors[23] and could be explained by the induction of tryptophan pyrrolase under this condition[24]. However, this explanation needs further investigations[25]. The discrepancies, observed by different authors, in the concentrations of plasma tryptophan in fasted and fed animals could be due to the age differences in the animals used, since younger animals are more sensitive to starvation.

Tryptophan is transported in blood mainly bound to serum albumin[26]. Certain hormones could modify the quantity of total tryptophan[27] as well as the percentage of the bound fraction of this amino acid[23,28]. In our experiments we observed a significant variation of free serum tryptophan, but the concentration of the total tryptophan remained unchanged. These results differ from those obtained by Nakhla et al.[4]. This could be due to differences in experimental conditions: i.e. fasting conditions. On the other hand, the increase in brain serotonin observed by these authors is confirmed by our results. It is known that the hydroxylation of tryptophan, which is necessary for the formation of brain serotonin, is dependent on the concentration of brain tryptophan but independent of the enzyme concentration[29]. In our experiments the increase in brain serotonin seems to be related to the parallel increase in serum free tryptophan and to the decrease of serum neutral amino acids. Furthermore these results seem to be in favor of a parallel increase of serum and brain tryptophan under the influence of calcitonin.

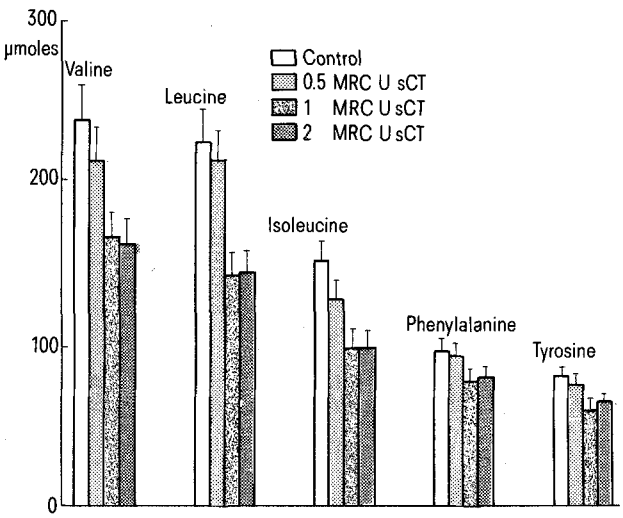

Figure 2. Decrease of the amino acid concentrations, 45 min after various amounts of calcitonin administration.

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