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DISPLACEMENT OF TRYPTOPHAN FROM ITS BINDING SITES SODIUM SALICYLATE

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Abstract. The concentration of free and bound tryptophan has been estimated in plasma, liver and brain of *Uromastix hardwickii,* before and after the intraperitoneal administration of sodium salicylate. Injection of the drug was found to enhance free tryptophan concentration, also the animal became slightly active. The pharmocological action of the drug, sodium salicylate has been correlated with the increase in free tryptophan concentration.

Among the various amino acids circulating in human plasma, tryptophan is the only amino acid significantly bounded to the plasma protein,[1] albumin.[2] The extent of tryptophan binding to protein has been studied by several techniques.[3-6] Some other substances like, sodium salicylate,[7,8] tetracyclines,[9] saccharin,[10] penicillin and secobarbitol[11] and phenothiazines[12] have been found to interact with human and bovine serum albumins. Administered salicylate has been found to displace tryptophan from its binding sites in plasma and liver of rates.[7,13] We studied the effect of sodium salicylate mainly on plasma and brain tryptophan levels to correlate it with the pharmocological behaviour of the drug. A local reptile *Uromastix hardwickii* was selected for the investigation. The present work describes:

(1) Determination of free and bound tryptophan and hence the degree of tryptophan binding to protein in plasma, liver and brain of the animal.
(2) Effect of administered sodium salicylate on the physiological level of tryptophan and the extent of tryptophan binding to protein.
(3) Assessment of other physical changes occurring in the animal.

Methods and Materials

Animals and Injection. Uromastix hardwickii were examined irrespective of age, sex and nutritional status of the animal. After a number of attempts it was possible to determine an effective dose of the drug for the animal, and 400 mg of sodium salicylate/kg body wt of the animal was injected intraperitoneally in 1 ml of NaCl (0.9% w/v). Control animal received an equal volume of NaCl.

Animals were killed after 0.5 hr of injection by stunning and cervical dislocations and their blood collected in heparinized centrifuge tubes. Blood was centrifuged to obtain plasma. Brain and liver of the animal were removed and stored at 0^{o} till used.

Estimation of Total Tryptophan. Estimations were made on whole plasma and 10% homogenates of liver and brain in NaCl (0.9%). A fluorimeteric method[14] was adopted for the estimation . The samples were treated with trichloroacetic acid/$FeCl_3$ (3×10^{-4} M solution of $FeCl_3$ in 10%, w/v, trichloroacetic acid) and centrifuged at 12000 rpm in a high speed centrifuge. Supernatant was incubated at 100^{o} in presence of formaldehyde. In this chemical process trichloroacetic acid releases tryptophan from its binding sites on proteins. Tryptophan then reacts with formaldehyde at 100^{o} to form a cyclic compound, which is later oxidized to norharman in presence of $FeCl_3$. The complete equation is given below.

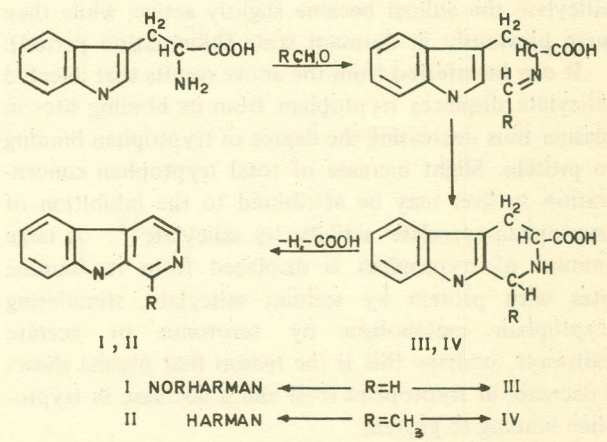

Fig. 1. The formation of harman and norharman from tryptophan

Norharman is a fluorescent compound which was estimated by EIL 27A-2 fluoremeter at excitation and emission wavelengths of 3655^{o} A and 4800^{o}A respectively.

Estimation of Free Tryptophan and Determination of Degree of Binding. 1 ml of plasma was dialysed and free tryptophan concentration was determined in the dialysate as above. Degree of binding was calculated as

TABLE 1. EFFECT OF SODIUM SALICYLATE INJECTION ON TRYPTOPHAN CONCENTRATION IN *Uromastix*. NUMBER OF ANIMALS ARE GIVEN IN PARANTHESES, CONCENTRATION IS EXPRESSED AS MEAN ± STANDARD DEVIATION.

Injection	Plasma (μg/ml) Total	Free	Bound	Liver (μg/g)	Brain (μg/g)
NaCl control (5)	9.98 ± 0.5	7.74 ± 0.8	22.4%	106.22 ± 11.6	9.18 ±4.5
Sodium salicylate experimental (5)	7.56 ± 0.3	6.91 ± 0.6	8.8%	110.93 ± 10.2	18.09 ± 1.3
P	< 0.001	> 0.05		> 0.10, < 0.3	< 0.005
% Difference	−24.25	−10.7		+4.43	+97

the % of total tryptophan present in bound form, i.e.

$$\frac{(\text{Total Tryptophan} - \text{Free Tryptophan}) \times 100}{\text{Total Tryptophan}}$$

Results and Discussions

It can be noted from the Table that administration of sodium salicylate decreases degree of tryptophan binding to protein and total tryptophan concentration in plasma. Slight increase in total tryptophan concentration was noted in liver while brain exhibited a considerable increase. Following the injection of sodium salicylate the animal became slightly active, while they were previously in dormant state (hibernation period).

It can be inferred from the above results that injected salicylate displaces tryptophan from its binding sites in plasma thus decreasing the degree of tryptophan binding to protein. Slight increase of total tryptophan concentration in liver may be attributed to the inhibition of tryptophan-pyrrolase activity by salicylate[13]. A large amount of tryptophan is displaced from its binding sites with protein by sodium salicylate, stimulating tryptophan metabolism by serotonin or acetate pathways, perhaps this is the reason that plasma shows a decrease in tryptophan level and a decrease in tryptophan binding to protein.

A pronounced increase in brain tryptophan concentration is important and may be attributed to the decreased tryptophan pyrrolase activity.[13] Tryptophan available to the tissues is that, present in free form, the equilibrium between bound and free form affects the influx of tryptophan in to the tissues. Influx of free tryptophan in to the brain and other tissues stimulates tryptophan metabolism. The metabolism of tryptophan through kynurenine pathway is checked due to inhibition of tryptophan pyrrolase activity hence it is mainly metabolised to serotonin. Serotonin is involved in smooth muscles and cerebral activity. Perhaps it is the greater synthesis of serotonin which activates the animal after salicylate administration.

It is a preliminary step to examine the neuro-pharmocological behaviour of salicylate. In this regard it will be important to determine actual serotonin concentration in brain after the administration of sodium salicylate. The neuro-pharmocological behaviour of other drugs like tetracyclines , phenothiazines, saccharin and pencillin etc, may also be investigated in a similar way.

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