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Some Factors Influencing Urinary Excretion of 3-Hydroxyanthranilic Acid. (29415)

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The increased excretion of 3-hydroxyanthranilic acid (3HAA) by a significant percentage of patients with rheumatoid arthritis has been reported by a number of independent investigators(1-3). This increased excretion has not been found in patients with other forms of arthritis or other types of disease, either infectious, granulomatous, inflammatory, degenerative, or neoplastic, with the exception of bladder carcinoma. 3HAA is an intermediary in the metabolism of the essential amino acid tryptophan by way of the kynurenine-nicotinic acid pathway(4). As far as is known, tryptophan is the only precursor of 3HAA(5). The cause for the increased excretion is not known. Prior studies have indicated that differences in dietary tryptophan intake are not responsible for the variations in 3HAA excretion(3). Among the possible mechanisms for the increased excretion are:

(1) An increased rate of gamma globulin turnover has been noted in some patients with rheumatoid arthritis(6,7). Since gamma globulin is relatively rich in tryptophan(8, 9), this increased turnover might lead to an increased production of 3HAA.

(2) Patients with rheumatoid arthritis ingest large quantities of salicylates. It has been shown that 3HAA oxidase, the enzyme responsible for 3HAA breakdown, is inhibited by salicylate *in vitro*(10). Therefore, it is possible that this inhibition occurs *in vivo* and thus prevents the breakdown of 3HAA.

(3) Patients with rheumatoid arthritis might, for reasons unknown, preferentially shunt tryptophan into the kynurenine pathway.

Whether these postulated mechanisms could indeed cause increased excretion of 3HAA was studied in a series of animal experiments.

Materials and methods. Six male white rabbits, each weighing approximately 2 kg, were maintained in metabolic cages on a standard diet and *ad lib* fluid intake. Urines were collected for consecutive 72-hour periods in the presence of concentrated sulphuric acid. After 2 control periods of 72 hours each, the rabbits were injected at 3-day intervals with formalin-killed *E. coli* as described by Abruzzo and Christian(11). Beginning the ninth week, the rabbits were injected with the bacteria daily. The animals were bled weekly. *E. coli* agglutinins and rheumatoid factor-like anti-gamma globulin antibodies were determined as previously described(11).

Another group of 6 similar rabbits were

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TABLE I. Urinary Excretion of 3-Hydroxyanthranilic Acid Following Sustained Immunization with *E. coli.*

	3HAA excretion (μg/24 hr)						
			Intervals after start of immunization				
Rabbit #	Control periods		9 days	18 days	36 days	70 days	82 days
142	45	51	70	56	70	57	70
143	60	68	69	41	59	53	25
146	57	77	79	93	82	84	—
147	88	—	106	101	103	100	65
148	109	105	128	118	—	70	120
150	107	98	Died				
Avg	78	76	90	82	78	73	70

maintained under the identical circumstances. After 3 control periods, they were subjected to the following procedures:

(1) 72-hour period of starvation. *Ad lib* water intake was allowed.

(2) 500 mg of l-tryptophan in saline was injected intravenously.

(3) Sodium salicylate, 50 mg/kg, was injected intraperitoneally daily for 14 consecutive days.

(4) 1 mg of 3HAA was injected intravenously in a single dose.

(5) 50 mg of hydrocortisone was injected intraperitoneally in a single dose.

The animals showed no ill effects from any of the procedures. Urines were collected for consecutive 72-hour periods in the presence of concentrated sulphuric acid. 3HAA determinations were done as described by Spiera(3). After each procedure, three 72-hour periods were allowed to elapse to permit 3HAA excretion to return to control levels.

Results. Urinary excretion of 3HAA in the immunized rabbits is shown in Table I. Results of the experiment in a representative animal are shown in Fig. 1. Immunization did not result in any significant changes in 3HAA excretion.

Table II summarizes results in the group of rabbits which received multiple treatments. Representative data from one animal are shown in Fig. 2. Starvation resulted in decreased 3HAA excretion, although 3HAA continued to be excreted in measurable quantities. Injection of 500 mg of tryptophan was followed by a moderate transient rise in 3HAA excretion. Two weeks of treatment with sodium salicylate resulted in no discernible change in 3HAA excretion. Intravenous administration of 1000 μg of 3HAA was not reflected by increased excretion of the metabolite. Injection of 50 mg of hydrocortisone was followed by an approximately 3-fold increase in 3HAA excretion.

Discussion. The extrapolation of results of experiments performed in one species to processes occurring in another species is not always valid. However, with that limitation

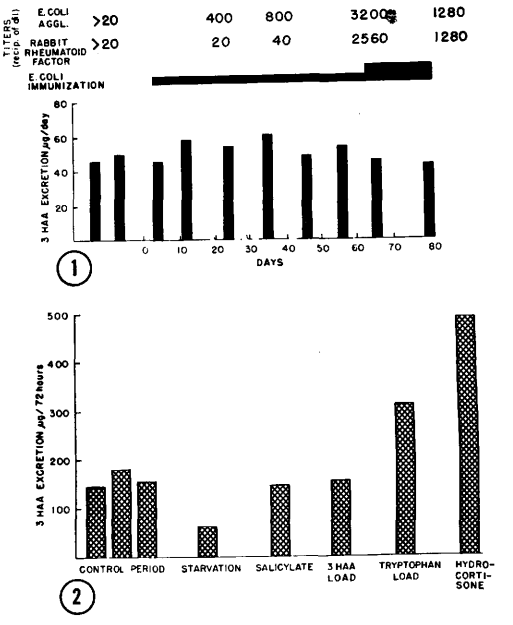

FIG. 1. Excretion of 3HAA in rabbits hyperimmunized with *E. coli.* Rabbit rheumatoid factor was measured by agglutination of rabbit isoagglutinin sensitized cells. The immunizing stimulus was increased after 60 days without change in 3HAA excretion.

FIG. 2. Effect of diet, salicylate, hydrocortisone, tryptophan and 3HAA loads on excretion of 3HAA in rabbits.

TABLE II. Urinary Excretion of 3-Hydroxyanthranilic Acid (μg per 24 Hr).

Rabbit #	Control periods		Fasting	Feed again	Sodium salicylate	Tryptophan, 500 mg I.V.	3HAA load	Hydrocortisone
1	93	75	27	80	88	91	86	244
2	51	47	31	—	46	80	46	93
3	82	89	37	76	93	163	45	252
4	27	37	30	—	40	80	105	165
5	90	84	29	86	85	107	77	200
6	41	32	40	—	40	81	63	112
Avg	64	61	32	81	65	150	70	176

in mind, several, conclusions may be drawn from the data obtained.

In the first experiment, the potency of the immune stimulus was evident by the production of high titers of both anti-*E. coli* antibodies and anti-gamma globulin factors. Failure of the rabbits to excrete increased quantities of 3HAA suggests that neither a sustained immune stimulus, the resultant increased turnover of gamma globulin, nor its other consequences, significantly influences excretion of 3HAA in this species.

The lack of effect of salicylate administration upon 3HAA excretion implies that inhibition of 3HAA oxidase by salicylate (noted previously in *in vitro* studies) does not occur sufficiently *in vivo* to influence 3HAA excretion. Further evidence in this direction comes from studies of patients with osteoarthritis and rheumatic fever who received large quantities of salicylates yet did not excrete increased quantities of 3HAA(1, 3).

Administration of hydrocortisone resulted in a sharp increase in 3HAA excretion, far greater than that resulting from administration of a tryptophan load. Feigelson and Greengard(12) showed that administration of 50 mg of hydrocortisone in the rat resulted in a 10-fold increase in hepatic tryptophan pyrrolase activity. Tryptophan pyrrolase is the enzyme responsible for the first step in the kynurenine-nicotinic acid pathway—the conversion of tryptophan to N-formyl kynurenine. Since the reaction is irreversible, a molecule of tryptophan that has gone through this step cannot be metabolized *via* other pathways. Although the enhanced 3HAA excretion noted experimentally with hydrocortisone treatment was probably mediated by an increase in tryptophan pyrrolase activity, it is unlikely that the excessive excretion in rheumatoid arthritis subjects is related to steroid therapy. The doses of cortisone, or its equivalent, used therapeutically are small in comparison to those required for the increase in enzyme activity and some of the highest 3HAA excretors have been patients not receiving steroid medications(3). It is interesting to note that, in addition to 3HAA, patients with rheumatoid arthritis also excrete increased quantities of kynurenine and hydroxykynurenine(2,13), the immediate precursors of 3HAA. Thus, it appears that the entire kynurenine pathway is more active in some patients with rheumatoid arthritis than it is in normals, and that the shunting of tryptophan into this pathway is mediated by something other than steroid therapy.

Summary. Excretion of a metabolite of tryptophan, 3-hydroxyanthranilic acid (3HAA), was studied in rabbits under varying conditions (immunization with gram negative bacteria, starvation, tryptophan loading, injection of 3HAA, sodium salicylate or cortisone). Starvation for 3 days resulted in a significant decrease in excretion of 3HAA, and injection of 500 mg of tryptophan (I.V.) was followed by an approximate doubling of 3HAA excretion. Immunization and injection with either salicylate or 3HAA (1000 μg) were not accompanied by significant changes in excretion of the metabolite. Administration of hydrocortisone, an agent known to increase liver tryptophan pyrrolase in other species, resulted in the maximal output of 3HAA observed. By inference, it is suggested that the higher excretion of 3HAA and other metabolites of tryptophan observed in some patients with rheumatoid arthritis may result from the preferential shunting of

tryptophan metabolism into the kynurenine pathway. Although the basis for such a shunt in rheumatoid subjects is not known, it is unlikely that adrenocortical steroids are responsible.

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An Indirect Hemagglutination Test for Diagnosis of *Mycoplasma pneumoniae* Infections. (29416)

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Serologic diagnosis of *Mycoplasma pneumoniae* (Eaton Agent) infection has not been a routine service of many diagnostic laboratories probably because no practical, relatively simple procedure has been available. Introduction of the indirect immunofluorescence test by Liu(1), whereby infected chick embryo lung sections are treated with serial dilutions of acute and convalescent sera and stained with fluorescein labeled gamma globulin, made possible a number of epidemiologic studies(2,3,4,5). However, this procedure is not likely to be adopted for routine diagnostic use as it is slow and requires elaborate equipment.

Chanock *et al* demonstrated that Eaton Agent could be grown on artificial media and suggested that colony impression smears might be substituted for chick embryo sections(6) but they later concluded that this method would not prove to be as satisfactory (7). More recently Chanock *et al* reported the use of a complement fixation test and found it to be at least 80% as sensitive as chick embryo immunofluorescence procedures (7). Other investigators have reported similar findings(8).

This paper describes a serologic test for *M. pneumoniae* which is based on Boyden's (9) findings that tannic acid-treated erythrocytes adsorb antigens and agglutinate when mixed with specific antiserum.

Materials and methods. Organism. The FH strain of *M. pneumoniae,* obtained on agar culture from Dr. R. M. Chanock, was used throughout the study.

Media. The culture media used were essentially those recommended by Chanock *et al*(6).

Antigen production and preparation. The entire contents of a 3-4 day old 60 mm agar plate were inoculated into 100 ml of broth per plate. Flasks were incubated for 4-5 days at 35°C and a second passage was made into fresh broth with a 10% inoculum. PPLO plate counts on the inoculum ranged from 10^3 to 10^4 colonies/ml. The second broth passage was incubated for 15 days and reduced to approximately 1/5 its original volume by dialysis against polyethylene glycol. The resulting concentrate was centrifuged for 1 hour at 30,000 rpm in a Spinco ultracentrifuge and the pellet was resuspended in a volume of phosphate buffered saline (pH 7.2) equal to ½ that of the original broth. Centrifugation was repeated under the same