was no change in the pulmonary glutathione S-transferase activity in vitamin A deficient rats. This is partly in disagreement with our findings as we have observed a significant decrease in pulmonary glutathione S-transferase activity. This could be due to strain variation. The exact mechanism, however, is not very clear. Moreover, the content of glutathione was significantly low in both liver and lung of these rats. Therefore, in addition to a decrease in the activity of glutathione S-transferase in the lung, the low content of glutathione in lung, where the normal level of glutathione is far less than that in liver, may result in prolonged failure to maintain an adequate intracellular supply of glutathione for conjugation and possibly be one of the factors towards enhanced susceptibility to lung cancer in vitamin A deficiency.

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The dietary origin of the urinary lignan HPMF

A. Coert, C.A. Vonk Noordegraaf, M.B. Groen and J. van der Vies[1]

Scientific Development Group, Organon International, NL-5340 BH Oss (The Netherlands), 7 December 1981

Summary. The influence of a synthetic diet devoid of plant fiber material on the occurrence of HPMF, a mammalian lignan, in rat urine was studied. The urine of rats on normal food contained significant amounts of HPMF. When the normal food was replaced by the synthetic diet the HPMF content fell to near-zero levels.

Two new compounds present in animal and human urine have recently been identified as trans-(±)-3,4-bis[(3-hydroxyphenyl)methyl]dihydro-2-(3H)furanone (HPMF) and a closely related butane-diol[2,3]. These substances are the first examples of lignans to be found in mammals, but their origin and biological importance are still unknown. Because of the cyclic pattern of its excretion in women[4] and the female vervet monkey[5], HPMF was first thought to originate from the gonads. From a recent study, however, it was concluded that HPMF and the diol are formed by intestinal bacteria[6].

Since lignans occur exclusively in higher plants we have, as a first step towards establishing the origin of these compounds, investigated the effect of the diet on the urinary HPMF levels in the rat. We have compared the effects of a normal rat diet and a semi-synthetic diet devoid of lignan and lignin-containing plant constituents. In addition we investigated the effect of gonadectomy. In this way we aimed to establish whether HPMF is formed from lignans, lignin or related substances derived from plants, or whether its formation is independent of the diet.

Material and methods. Adult male and female rats of Wistar origin were housed individually in metabolism cages, and fed normal rat food (RMH-B, Hope Farms, Woerden, The Netherlands) for 8 days. They were then changed to a synthetic diet of the following composition: casein (22%),

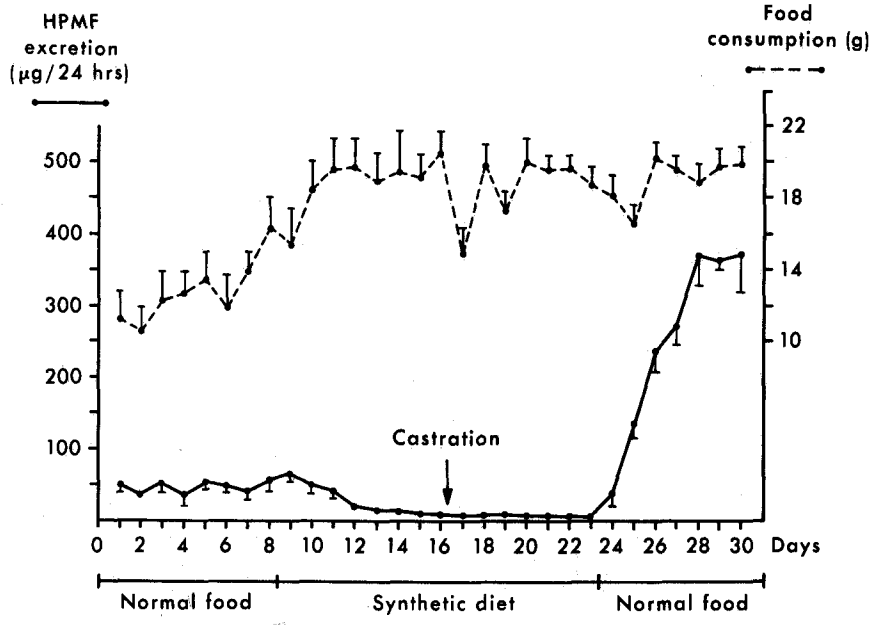

Figure 1. Food consumption and urinary HPMF excretion of male rats fed normal rat food and a synthetic diet. The values are given as means (n=6). The bars represent SEM.

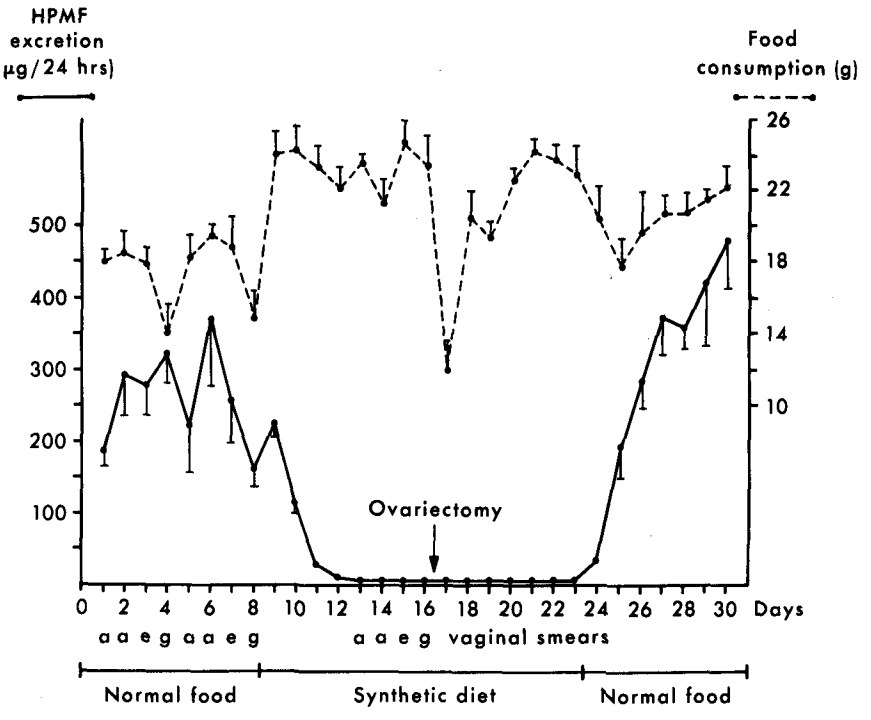

Figure 2. Food consumption and urinary HPMF excretion of female rats fed normal rat food and a synthetic diet. The values are given as means (n = 6). The bars represent SEM. Vaginal smear code: a = dioestrus, e = prooestrus and g = oestrus.

corn starch (6%), glucose (63.1%), sunflower seed oil (1.3%), dairy butter (1.7%), $CaCO_3$ (1.7%), $CaHPO_4$ (0.8%), KH_2PO_4 (1%), MgO (0.3%), d,l-methionine (0.2%), choline chloride, 50% (0.4%), vitamin premix in glucose (1%), and trace elements premix in glucose (1%), supplied by Hope Farms, Woerden, The Netherlands. The synthetic diet was maintained for 15 days and then replaced by normal rat food again. After 8 days on the synthetic diet male and female rats were gonadectomized.

Vaginal smears were taken daily up to the time of ovariectomy. 24-h urine samples were collected for the duration of the experiment and frozen until analyzed. Urine samples were hydrolyzed with suc d'*Helix pomatia* for 24 h, after which the phenolic fraction was separated[7] and silylated. The HPMF content was determined by GLC of the HPMF-bistrimethylsilyl-ether on an OV-17 column.

Results and discussion. Figures 1 and 2 show that in both male and female rats the urinary HPMF content fell to near-zero levels with the synthetic diet, and rose above the initial levels when the rats were returned to normal food. Gonadectomy during the periods on the synthetic diet had no direct influence on HPMF excretion.

It has recently been reported that germ-free rats do not excrete HPMF or the diol, but no details were given of the diet[6]. This finding led to the conclusion that HPMF is formed by the intestinal bacteria[6,8] and that it is probably not derived from a lignan or a lignan precursor in the food, because structurally related plant lignans are optically active and HPMF isolated from urine is racemic[9]. Our results, however, strongly suggest that, in the rat, the mammalian lignan HPMF is directly derived from plant constituents in food. As pointed out above plant lignans are not likely to be the source of HPMF. However, lignin is a possible source because it is optically inactive and it contains structure elements which can lead to the formation of HPMF[10]. After ingestion lignin may be broken down in the gut, probably by intestinal bacteria, either directly to HPMF or to its precursor(s). In the latter case the degradation products may be converted into HPMF and the diol elsewhere in the body.

The following observations implicate an involvement of the pituitary and/or the gonads in the production or the regulation of the production of HPMF: a) HPMF follows a cyclic pattern in the woman[4] and the female vervet monkey[5]: b) patients undergoing ovarian stimulation with FSH and LH (Pergonal®) showed a marked rise in the excretion of HPMF during the post-ovulatory phase[4]; c) during pregnancy HPMF excretion more or less parallels the HCG levels[4]; d) ovariectomy of rats on a normal diet caused an increase in HPMF excretion (unpublished); and e) in the present study gonadectomy during the synthetic diet period had no influence on HPMF excretion, but on returning to the normal diet, the levels rose above the initial levels in both males and females. This rise was particularly marked in the males.

All these data indicate a role of gonadotrophins in the regulation of the production of HPMF from dietary precursors. Suppression of the pituitary gonadotrophin secretion, by treatment of rats with an oestrogen or a progestagen, and subsequent substitution with HCG could give more information on the possible regulation of the production of HPMF by gonadotrophins.

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