Reports on the vitamin A requirements for the normal albino rat vary widely. However, it appears that 250 IU/week is about twice the normal requirement to maintain the integrity of mucous membranes[8]. The relative inhibition of estrogen-induced endometrial metaplasia by these moderate doses indirectly supports the contention of McCULLOUGH and DALLDORF[5] and CRAMER[6]; i. e. that vitamin A deficiency is an etiological factor in producing these lesions. However, it is certainly not proof of this hypothesis since uterine keratinizing metaplasia never develops in bilaterally ovariectomized rats maintained on vitamin A-free diets for long periods of time, e.g. group 1 of the present investigation. Furthermore, estrogen-treated rats develop keratinizing metaplastic lesions without other gross signs of vitamin A deficiency (unkempt fur, dyspnea, etc.). The absence of heteroplastic changes in the uteri of the ovariectomized, vitamin A-deficient rats supports the suggestion of Bo[9] that a deficiency of this vitamin is not an important factor in producing stratified squamous metaplasia in the uterus of the rat.

Incidence and extent of metaplastic lesions observed in the uteri of the animals within the five experimental groups

Group	Treatment	No. of rats	No. of rats with uterine metaplasia	Extent of lesions [a, b]
1	Ovariectomized Vitamin A-deficient diet	7	0	—
2	Ovariectomized Vitamin A-deficient diet Estrogen	11	10	+++ (4); ++ (5); + (1)
3	Ovariectomized Vitamin A-deficient diet Estrogen 250 IU vitamin A	9	2	+ (2)
4	Ovariectomized Vitamin A-deficient diet Estrogen 100,000 IU vitamin A	15	12	+++ (4); ++ (7); + (1)
5	Ovariectomized Vitamin A-deficient diet 100,000 IU vitamin A	6	0	—

[a] + indicates one to several metaplastic foci of the glandular or luminal epithelium; ++ indicates more numerous small metaplastic lesions; +++ indicates very extensive metaplastic lesions involving the entire or nearly the entire endometrium.
[b] Numbers in parentheses indicate number of animals with a lesion of this extent.

Although the importance of an adequate supply of vitamin A for maintaining most epithelial membranes is well established, evidence is accumulating which indicates that high doses of vitamin A are just as deleterious as an absence of this vitamin. SHERMAN[10] found that by adding large doses of vitamin A to culture media, a significant decrease in the mitotic index of cultured skin, cornea, and trachea was observed. LAWRENCE, BERN, and STEADMAN[11] reported epithelial atrophy of hamster cheek pouch epithelium after applications of high concentrations of vitamin A. The injurious effects of excess vitamin A were recorded also by FELL et al.[12]. They conjectured that the severe effects on tissue explants were related to the influence of excess vitamin A on the permeability of certain subcellular membranes. Since excess estrogen stimulation also causes degeneration of the normal uterine columnar epithelium followed by reparative stratified squamous metaplasia[13], it is not surprising that when the two were administered simultaneously the percentage of animals with uterine changes was high (group 3). In these experiments, however, atrophic or metaplastic changes were not produced in the uterus when large doses of vitamin A were given alone (group 5). The results show that certain levels of vitamin A and estrogen are required to maintain normal endometrial morphology and that a systemic imbalance of these substances can cause pathological alterations in the reproductive tract of the female rat.

Zusammenfassung. An der Ratte wurde der Einfluss von oral verabreichtem Vitamin A auf die Östrogen-induzierte Metaplasie der Uterusschleimhaut untersucht. Kleine Dosen von Vitamin A (250 IE pro Woche) sind zur Vermeidung dieser Metaplasie wirksamer als grosse Dosen von Vitamin A (100000 IE pro Woche).

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Effect of Agar on Bradykininogen Levels and Esterolytic Activity in Rat Plasma

Previous results[1] have shown that the increased capillary permeability observed during passive cutaneous anaphylaxis (PCA)[2], induced by heterologous antibody and antigen in the rat, could be suppressed in animals which had received an intravenous injection of starch or agar. This inhibitory effect was obtained in spite of undiminished skin histamine levels and of an unimpaired sensitivity of agar-treated animals to histamine release by compound 48/80, dextran or ovomucoid. These findings led to the conclusion that the release of endogenous histamine probably plays no major role in this type of passive anaphylactic reaction. A similar conclusion, extensive to

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5-hydroxytryptamine as well, has been arrived at by other workers[3]. Bradykinin, a polypeptide arising through the enzymatic cleavage of a plasma protein (bradykininogen), has marked effects on skin capillary permeability[4]; it has also been shown to arise during systemic anaphylaxis in the rat[5]. The present study was undertaken in order to discover the possible participation of bradykinin in PCA reactions by examining whether the increased resistance to this phenomenon in rats treated with agar[1], was accompanied by changes in plasma bradykininogen levels. Since other authors[6,7] have indicated that agents which release bradykinin are also able to split arginine ester derivatives, it was considered of interest also to examine the esterolytic activity of the plasma of agar-treated rats, on *p*-tosyl arginine methyl ester (TAME).

Female Wistar rats (150–200 g) were intravenously injected with 0.5 g/kg of agar (Difco). Like the product employed before[1], the polysaccharide had been partially hydrolysed by reflux boiling in 0.1*N* acetic acid until, while retaining its activity, the neutralized hydrolysate no longer formed a gel when kept at 37°C. In this way the danger of an embolism following intravenous injections was avoided, and except for a transient prostation, the product appeared to be well tolerated by the animals. Controls were prepared by equivalent injections of 0.9% saline. 50 min after treatment, blood was withdrawn from the abdominal aorta of the ether-anaesthetized animals, using a siliconized syringe containing sodium oxalate (2 mg/ml of blood). Plasma was obtained by centrifugation and its bradykininogen content determined[8], using crystalline trypsin (Worthington), to convert bradykininogen into bradykinin. Esterolytic activity on TAME was also measured[9].

The Table shows that the average value of bradykininogen in the plasma of control rats was 5.16 units/ml; this agrees with previous findings[8]. After the animals had been treated with agar, their plasma bradykininogen dropped markedly, reaching, on an average, a value of 87% lower than that of the controls. A bradykininogen depleting effect of agar was thus demonstrated. In additional experiments, fresh normal rat plasma was mixed with agar in amounts sufficient to give a final concentration equal to the one calculated to exist in the plasma of the treated animals; when assayed immediately after mixing, normal bradykininogen values were found in such samples. This indicates that agar did not interfere with the action of trypsin on bradykininogen, an essential step in the procedure employed for the assay of this substrate. The effect of agar on bradykininogen levels could result from an in vivo activation of an enzyme system capable of attacking this bradykinin precursor in plasma. As shown in the Table, the activity of plasma TAME esterase was significantly increased in agar-treated rats. This result suggests that agar is able to promote the activation of an enzyme system which, while sharing with normal plasma esterase its ability to split TAME, has in addition the faculty of attacking bradykininogen. When considered in relation to previously presented data[1], these results indicate that the inhibition of PCA in agar-treated rats may be due to a depletion of bradykininogen, and they point towards a possible participation of bradykinin in this anaphylactic reaction.

A significantly increased level of esterolytic activity on TAME was also observed in vitro when fresh rat plasma was incubated for 5 min at 37°C with 10 mg/ml of agar. This treatment also leads to the formation of anaphylatoxin[10], a potent histamine releasing agent in certain species. Further work is planned to test whether the parallelism between conditions leading to the appearance of a histamine releasing principle on the one hand, and the breakdown of bradykininogen on the other, has more than a mere coincidental significance.

Little is known about the chemical nature of the interaction of agar with plasma proteins; it seems possible that, in activating a plasma esterase system, agar acts by an adsorption mechanism through which an inhibitor, having a high affinity for a long chain polysaccharide like agar, is removed from the medium. It is interesting to note that an activation of plasma esterase by heparin, a compound having a certain structural resemblance to agar, has been noted[11].

Effect of intravenously administered agar on bradykininogen levels and esterolytic activity of rat plasma

	Means + standard errors		Significance
	Controls	Agar treated	*P*
Bradykininogen (units/ml)	5.16 ± 0.61 (10)	0.85 ± 0.08 (8)	< 0.001
Activity on TAME (μmoles hydrol./ml/h)	76.20 ± 3.40 (18)	102.60 ± 4.70 (10)	< 0.001

Figures within parentheses indicate number of animals used. Significance (*P*), determined by Student's 't' test.

Zusammenfassung. Bei der Ratte führt intravenös injizierter Agar zur Senkung des Bradykininogengehaltes, Erhöhung der *p*-Tosyl-L-Arginin-Methylester (TAME)-Spaltfähigkeit, ferner zur Aufhebung der Sensibilität der Rattenhaut für Permeabilitätsstörungen durch passive kutane Anaphylaxie. Danach und nach in-vitro-Versuchen besteht die Möglichkeit, dass Bradykinin bei solchen Hautreaktionen eine Rolle spielt.

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