

the thread, were fixed and stained for histologic study.

The results are indicated in Table I. It is seen that a total dosage of 1 mg of testosterone propionate given under these conditions failed to produce either hypertrophy of the corpora lutea or deciduomata. Deciduomata were formed in 2 of 5 animals given 2.5 mg and in all animals receiving 5 or 10 mg, but hypertrophy of the luteal cells was found only when 20 mg of the hormone were given. This enlargement is illustrated by the representative camera lucida drawings in Fig. 1, where it also can be compared with cells of various other types of corpora lutea.

Uniform results were obtained with prolactin. With 600, 1200, and 2400 I.U., the uteri reacted with deciduomata in 9 out of 10 rats but in no instance was there any enlargement of the corpora.

It is significant that the combined adminis-

tration of 5 mg of testosterone propionate and 400 I.U. of prolactin produced enlarged corpora, because at this dosage level neither hormone by itself produced this effect.

Summary. The administration of testosterone to rats, provided the initial injection is given during late estrus, leads to an enhanced and prolonged function of the corpora lutea, so that deciduomata can be produced by traumatization of the uterus. The minimal total dosage necessary to produce this effect is between 2.5 and 5.0 mg, given over a period of 10 days. If it is increased to 20 mg there is, in addition, a marked hypertrophy of the corpora lutea cells, similar to that of normal pregnancy.

A similar administration of prolactin in total dosages of from 600 to 2400 I.U. produces active corpora lutea, as shown by deciduoma formation, but there occurs no hypertrophy of the luteal cells.

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Inhibition of Experimental Liver Cancer in Rats by Addition of an Adsorbent to the Diet.

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The inhibiting influence of yeast, liver, rice bran and their extracts on the carcinogenicity of the azo dyes has been studied recently by Sugiura and Rhoads¹ who reported that only part of the protective factor in yeast could be extracted with ether. We have observed that the relatively indigestible fractions of the foods mentioned, when placed in an aqueous solution of the carcinogenic dye known as butter yellow (paradimethylaminoazobenzene), adsorb considerable amounts of the dye *in vitro*. It therefore occurred to us that part of the protective influence of these dietary constituents on butter yellow cancer might be due to reduced absorption of the carcinogen and that the incidence of this type of experimental tumor might be greatly reduced by

the inclusion in the diet of a good, non-toxic, indigestible adsorbent, such as the clay, montmorillonite.*

The present report deals with the adsorption capacity for butter yellow of several materials which might be suitable for inclusion in experimental diets containing this carcinogen, together with preliminary results on feeding one of these (montmorillonite) on the incidence and course of butter yellow induced tumors.

The adsorption capacity of the various materials for butter yellow, determined by the

* Montmorillonite ($(\text{Mg}, \text{Ca})\text{O} \cdot \text{Al}_2\text{O}_3 \cdot 5\text{SiO}_2 \cdot n\text{H}_2\text{O}$) is a natural white clay used by refiners for removing undesirable substances from foods and petroleum products. That used by us was mined in Gonzales County, Texas, and generously donated by the Milwhite Company of Houston, Texas.

¹ Sugiura, K., and Rhoads, C. P., *Cancer Research*, 1941, 1, 3.

general method of Fantus² for basic dyes, is expressed in milligrams of dye adsorbed by 10 g of the water insoluble material before and after subjection to 12 hours of peptic and 18 hours of tryptic digestion at 37°C.

	Before digestion mg	After digestion mg
Dried brewer's yeast	8.61	7.54
Dried beef liver	8.74	8.50
Rice bran	9.05	8.40
Unpolished rice flour	5.26	4.00
Montmorillonite	954.00	472.00

Since montmorillonite is practically insoluble except in strong NaOH or HCl solutions, the effect of digestion on its adsorption capacity is probably due to initial saturation of its surface with enzymes or other protein-like substances in the digestive fluids.

The feeding experiments were carried out on 40 young rats of the Wistar strain which were divided into 2 groups similar in number, sex, age, and weight. The control group was fed butter yellow according to the method used in the experiments of Sugiura and Rhoads.¹ The treated group received a diet containing 10 parts of the basic carcinogenic ration and 1 part (by weight) of montmorillonite. This adsorbent clay was assayed for its riboflavin content by the method of Swaminathan³ and found to be free of this vitamin, an important feature since the protective effect of yeast, bran, and liver has been attributed partly to the high riboflavin content of these foods.⁴

During the period from the 60th to the 65th day of dieting, the animals of each group were placed in individual metabolism cages and the amount of butter yellow excreted in

the urine was determined by colorimetric method. It was found that the animals receiving montmorillonite in their ration excreted about 53% less butter yellow in the urine than did the rats receiving none of the added adsorbent.

The results of feeding the carcinogenic diets for a period of 175 days are as follows:

1. Eighteen of the 20 control animals developed tumors which were capable of detection by palpation at the end of 175 days or by macroscopic examination at autopsy before this time. Only one of the 20 montmorillonite-fed rats developed a palpable tumor within the 175 days.

2. While only 8 of the 20 control rats survived the 175 days, 17 of the 20 montmorillonite-fed animals survived this period.

3. The average weight gain per animal during the first 100 days of the experiment was 74 g for the control group and 54 g for the montmorillonite-fed group.

4. The eyes, hair, and activity of the rats revealed no marked vitamin deficiency in either group.

Summary. The relatively indigestible fractions of yeast, liver, and rice bran efficiently adsorb butter yellow (paradimethylaminoazobenzene) and thus remove this carcinogen from solution *in vitro*. In order to determine whether an adsorbent alone might confer protection against carcinogenesis, a riboflavin-free adsorbent clay, montmorillonite, was added to a diet containing butter yellow and fed to 20 young rats. During 175 days of feeding, only one of the 20 animals developed a palpable liver tumor, whereas 18 of 20 control rats developed such a tumor. That a considerable portion of the carcinogen was eliminated by adsorption upon the clay was indicated by the observation that the rats receiving the clay excreted 53% less carcinogenic dye in the urine than did the animals receiving no montmorillonite.

² Fantus, B., *J. A. M. A.*, 1915, **64**, 1838.

³ Swaminathan, M., *Ind. J. Med. Res.*, 1942, **30**, 23.

⁴ Kensler, C. J., Sugiura, K., Young, N. F., Halter, C. R., and Rhoads, C. P., *Science*, 1941, **93**, 308.