Table 2. Correlation between sex pheromone production and mating. The numbers in parentheses are percentages.

No. tested	Sex pheromone production			
	Presence		Absence	
	Mating	No mating	Mating	No mating
	Unoperated controls			
30	12	11	0	7
	Sham-operated controls			
23	10	11	0	2
	Allatectomized females			
28	4	6	2	16
	Totals			
81	26 (48.1)	28 (51.9)	2 (7.4)	25 (92.6)

were provided. Nearly all of the 54 females in Table 2 that produced pheromone were "courted" by males immediately after the latter were introduced, whereas this behavior was provoked by only one of the 27 females that failed to produce the pheromone. The high correlation between pheromone production and successful mating is evident in Table 2. In addition, it is noteworthy that the two females that mated in spite of the failure to produce pheromone began to produce small quantities of pheromone some weeks later. Therefore, they may have done so during the test period.

The implantation of corpora allata into previously allatectomized females can result in the recovery of the ability to produce pheromone. In these experiments, each female received by injection into the anterior part of the abdomen four corpora allata taken from adult females 1 to 3 days after the imaginal molt. Two of six animals which received implants 8 to 10 weeks after the imaginal molt showed a strong and sustained recovery of pheromone production beginning 10 and 16 days after implantation. One of these two animals subsequently mated. An older group of 12 animals (implants received 9 to 14 weeks after the imaginal molt) showed no recovery of the ability to produce pheromone. All died 2 to 6 weeks after the operation. Failure to recover may be related to the age of these animals at implantation, for in the absence of corpus allatum stimulation during this prolonged period, the tissue producing the pheromone may have degenerated to such an extent that recovery was no longer possible. Experiments on a series of young adult females (5 to 6 weeks of age) are in progress. Preliminary results show recovery of pheromone production in three of seven animals.

These findings suggest several lines of investigation for future consideration. Of particular interest is the question as to whether pheromone production is directly or indirectly stimulated by the corpus allatum hormone. Preliminary experiments argue against an intermediary role for the ovary, since the removal of the ovaries from last-instar nymphs had no effect on the subsequent production of pheromone. It is also necessary to decide whether pheromone production is the only aspect of the female's mating behavior that is affected by allatectomy. Finally, the results draw attention to the possibility that female sex pheromone production is subject to endocrine control in other species and orders of insects (*8*).

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Nutritional Value of Chemically Modified Corn Starches

Abstract. Male and female weanling rats were fed daily 5 g of a balanced diet supplemented with 1 or 2 g of corn starch, oxidized corn starch, corn starch phosphate, or hydroxyethyl corn starch. Commercially modified corn starches produced the same weight gain as normal corn starch during 21 days of feeding. In contrast to commercial starches, a very highly oxidized laboratory-prepared starch produced a lower weight gain during the same period.

Since increasing amounts of modified starch are being produced to meet requirements for industrial and food uses, it seemed desirable to determine whether slightly modified corn starch might be somewhat more digestible and give higher caloric value than unmodified corn starch.

The nutritional value of starches has been examined by several investigators. Booher *et al.* (*1*) showed that wheat, rice, corn, and waxy maize starches are more digestible in rats than the starches of arrowroot, white potato, and sago palm. Sakurai *et al.* (*2*) found that raw or cooked starches from most cereal grains are effective in producing growth in rats when they are fed as 75 percent of a balanced diet. White potato starch, ball-milled for 250 hours, is equally effective in producing growth, but raw untreated white potato starch is less effective. Jelinek *et al.* (*3*) stated that raw white potato starch is a poor nutritional carbohydrate when fed as 73.6 percent of the diet of weanling rats, but that autoclaved, modified, or ground potato starch is more fully utilized. Booher *et al.* (*1*) showed that modification which produces hydration of, changes the chemical nature of, or disrupts the starch granule makes the carbohydrate a better food substance.

The feeding procedure used was that designed by E. E. Rice *et al.* (*4*) which is based on the theory that food is used for energy to the greatest extent until minimum energy requirements are met. It has been established that the growth of weanling rats varies in proportion to the metabolically available energy of a food when a minimal diet is fed.

Corn starches used as diet supplements were commercial products: corn starch oxidized by 6 percent (wt./wt.) of chlorine, intrinsic viscosity 0.25, carboxyl content 20 meq/100 g of starch; corn starch oxidized by 2.5 percent (wt./wt.) of chlorine, intrinsic viscosity 0.45, carboxyl content 7 meq/100 g of starch; hydroxyethyl corn starch, 0.11 degree of substitution; corn starch phosphate, 0.5 to 0.9 degree of substitution (two samples); and a laboratory sample of corn starch oxidized by 2 equivalents of hypochlorite per D-glucose unit, 43.2 percent (wt./wt.) of chlorine. This very highly oxidized noncommercial starch was prepared only to determine whether

Table 1. Weights gained by rats in 21 days on different types of starch. D.S., degree of substitution.

Type of corn starch	Supplement level	
	1 g	2 g
	Weight gain (g)	
Group 1		
Corn starch	33.7	41.4
a) Corn starch oxidized by 6 percent (wt./wt.) chlorine	33.6	45.0
b) Hydroxyethyl corn starch, 0.11 D.S.	31.6	42.3
c) Corn starch oxidized by 2 eq of hypochlorite, 43.2 percent (wt./wt.) chlorine	18.9	29.7
Group 2		
Corn starch	32.8	43.2
d) Corn starch oxidized by 2.5 percent (wt./wt.) chlorine	35.2	46.8
e) Corn starch phosphate, 0.5 to 0.9 D.S.	36.5	43.5
f) Corn starch phosphate, 0.5 to 0.9 D.S.	33.8	45.2

any effect in weight gain could be produced by feeding a seriously overoxidized product.

Weanling rats (Wistar-Purdue strain) were fed daily 5 g of a highly nutritious basal diet (*5*) supplemented by 1 or 2 g per day of the starch products under investigation. The basal diet (5 g/day) was sufficient to produce a weight gain of 4 to 8 g per week during 4 weeks.

The experiment was conducted in two parts, in each of which nine diets were fed. These were as follows: one basal diet of 5 g/day; four supplemented diets containing 5 g of basal diet and 1 g of a starch product, or a total of 6 g/day; and four supplemented diets containing the basal diet and 2 g of a starch product, or a total of 7 g/day.

The nine diets were arranged in a randomized incomplete block design (*6*), and each diet was replicated four times. Equal numbers of male and female weanling rats were grouped (three litter mates per block, three blocks per replication), individually caged, and fed 5.0 ± 0.1 g per day for 7 days. Water was freely supplied. At the end of this period the animals were weighed and either continued on the basal diet or changed to diets composed of basal diet mixed with 1 or 2 g of starch product supplement. Animals were weighed again after 3, 7, 14, and 21 days of supplementation.

Responses to supplementation were so uniform that 7-day weights reflected the increased caloric intake as definitely as weights at 14 days or 21 days. The weight gains at 21 days for the rats in the two groups of the experiment are presented in Table 1. The data for each group were analyzed in a 4 by 2 factorial design: four types of starch fed at two levels. This anaylsis permitted evaluation of the effect of the amount of supplement, the type of supplement, and the interaction of type and amount.

All the commercial starches produced weight gains similar to corn starch (Table 1). However, starch *c*, heavily oxidized and degraded by 2 equivalents of hypochlorite per D-glucose unit (43.2 percent, wt./wt., of chlorine) had a low nutritional value since the average weight gain produced by feeding this starch was significantly lower than all the others. The average weight gains produced by supplements of 1 and 2 g of this starch were not very much different from that produced by the basal diet (a gain of 26.7 g during 21 days).

The average weight gain produced by all 2-g supplemented diets was significantly greater than that produced by 1-g supplemented diets, the difference being about 10 g per rat in 21 days. There was no interaction between the amount of supplement and type of starch; this is shown by the fact that doubling the amount of supplement increased the gain about equally for all the starch products. There was close agreement between the weight gains reported for corn starch in groups 1 and 2. This similarity allowed a comparison of weight gain between products in the two groups. Heavily oxidized noncommercial starch (*c*) induced diarrhea after the 2nd day of supplementation. Hydroxyethyl starch produced a mild diarrhea.

Autopsies performed on one rat from each of the 2-g supplemented diets disclosed that rats fed heavily oxidized starch *c* had a marked dilation of the colon. The other animals appeared to be normal (*7*).

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Maintenance of Normal in situ Chromosomal Features in Long-Term Tissue Cultures

Abstract. Clonal isolates from a rapidly proliferating fibroblast-like derivative have retained the classic diploid chromosome relationship over a period of many transplant generations. The readily identified members of the 11 pairs of chromosomes, including the sex chromosomes (X_1 and X_2), have aided in localizing minute structural alterations within recloned diploid and aneuploid sublines.

An extensive search to isolate stable diploid tissue cultures of the Chinese hamster (*1*), which continually display the chromosomal state noted *in situ* (*2*), has been wholly successful. The twenty-eighth growth in the series, stemming from adult female fibroblast-like derivatives (FAF-28), possessed the many desired features that characterize the classic diploid state (Fig. 1). The strain was derived from a population of normal cellular components that had infiltrated the peritoneal cavity of an animal bearing tumor CH-38MC, a 3-methylcholanthrene-induced fibrosarcoma. This scheme to initiate cultures was routinely employed, since tetraploid tumor cells always failed to attach to the surface of the flask, leaving normal cellular infiltrates to proliferate for varying lengths of time and, in some cases, without undergoing the eventual shift toward aneuploidy. Generation times gradually shortened as sublines progressed into the tenth month, at which time single-cell cloning trials were conducted in an effort to isolate the increasing number of classic tetraploids. Current parental and clonal derivatives proliferate very rapidly (14 hours or less) in a variety of chemically defined media prepared with whole serum (*3–5*).

Plating efficiencies of three classic diploid and one subdiploid variant, isolated by a modification of procedures described by Puck *et al.* (*5*), ranged from 20 to 40 percent, 7 to 12 days after 60-mm petri dishes were seeded with 500 to 2000 single cells, and in the absence of subsequent and recommended changes of the medium. Precautions regarding serum toxicity and prescribed handling of glassware were neglected during these preliminary trials. FAF-28 exhibits very few spontaneous chromosome breaks. Aneuploidy, that is, a ±1 chromosome deviation from the euploid number, rarely exceeds 20 percent of the cells seen in division, as in the case of intact bone marrow, regenerating liver, and corneal epithelium. Tetraploidy in FAF-28 has fluctuated from 1 to 25 percent of the mitotic population and has consisted primarily of classic tetraploids arising from endoreduplication. It appears that repeated trypsinization of rapidly proliferating classic diploid cells causes dividing metaphases to undergo restitution when suddenly detached and transferred to the new culture flask. Somatically paired homologues are seen more frequently early in the next transfer generation.

Although the later parental FAF-28 sublines were characterized by rigid classic diploidy and tetraploidy, single-cell clonings yielded an unexpected array of aneuploids and even near-triploids. The second cloning trial failed to reveal triploids. On both occasions, classic tetraploid cell types failed to clone. Parental-like classic diploid sublines were repeatedly isolated