in the film of bovine albumin used to obtain the spectrum illustrated.

That this same sample of bovine serum albumin was still in the native form is demonstrated by the data in Table 1. The protein film, dissolved in phosphate buffer at pH 7.6, depressed the optical absorption of methyl orange, as is characteristic of native albumin. Furthermore, the degree of depression, in comparison with that obtained with the control, untreated sample, was roughly in proportion to the quantity of protein. Clearly then, no significant fraction of the albumin was denatured by deposition as a film, drying, or infrared radiation.

TABLE 1

TEST OF NATIVE CHARACTER OF DRIED PROTEIN FILM BY EXAMINATION OF ITS EFFECT ON SPECTRUM OF METHYL ORANGE

Wave length (A)	Depression of optical density by protein	
	From dried film*	Untreated†
4,600	0.063	0.099
4,700	0.097	0.138
4,800	0.117	0.165
4,900	0.134	0.175
5,000	0.123	0.167

* Contained 8 mg of protein.

† Contained 12 mg of protein.

In view of these experiments, there would seem to be little room left for doubt that native, globular proteins contain an amide-type carbonyl group.

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The Effect of Choline-Deficiency on Uterine Activity of Rats

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Peet and Sampson (*7*) reported recently that two post-partum albino rats, one nursing young and one nonlactating, maintained for 39 days on a choline-deficient diet, were found at autopsy to lack completely contractility of the uterus and to have partial atrophy of the uterus and ovaries. Two controls, both nursing young, which received respectively 30 mg and 200 mg of choline every second day, showed normal uterine activity. A yellow, oily condition of the hair on the rats receiving the choline supplements was also noted.

The work reported here was undertaken on a larger group of animals to confirm these observations if possible.

Nine Carworth Farms adult virgin female rats of the Wistar strain were used unmated and nine similar rats were mated. Two of the mated rats died during pregnancy and seven delivered litters, six of these nursing from 7 to 10 young apiece. All virgin rats were placed on the experimental diets at the same time, and each mother was given the experimental diet following delivery of her litter. The diets were distributed among the animals as follows: 3 virgin females on Purina Dog Chow; 3 virgin females, 3 nursing females, and 1 postpartum nonlactating female on the choline-deficient diet; and 3 virgin females and 3 nursing females on the choline-deficient diet plus a choline supplement.

TABLE 1

EFFECTS OF CHOLINE-DEFICIENCY AND LACTATION ON BODY WEIGHT AND ON OVARIAN AND UTERINE WEIGHTS

Rats & diet	No. in group	Average body weight change	Average ovarian weight	Average uterine weight
Nonlactating virgin rats				
Chow controls	3	+ 38 gm	68 mg	476 mg
Diet controls	3	+ 37 gm	66 mg	445 mg
Choline-deficient	3	+ 39 gm	59 mg	421 mg
Nonlactating postpartum rat				
Choline-deficient	1*	+ 56 gm	64 mg	438 mg
Nursing rats				
Diet controls	3*	− 29 gm	74 mg	244 mg
Choline-deficient	3*	− 40 gm	69 mg	291 mg

* One animal from each group was removed from the yeast-casein supplement during the last 14 days of the experiment.

The choline-deficient diet developed by Hegsted and associates (*6*) was used in this work as it was in the work by Peet and Sampson.

The 30 mg choline supplement given every second day was chosen for all control animals in preference to the 200 mg since that amount more nearly approximates physiological levels of the substance (*1, 2, 4, 8*). The additional supplement (*7*) of 1 gm dried brewer's yeast and 5 gm extracted casein was provided every second day to all rats on the basal diet except 3, which were deprived of it completely for the last 14 days of the experimental period in an effort to intensify the choline-deficiency.

Vaginal smears were taken daily and rats were weighed at weekly intervals. All rats were killed by cerebral concussion on the 39th day with the exception of the animals on the Dog Chow diet, which were killed at later intervals when control uteri were desired. At the time of experimentation the ovaries and uteri were rapidly excised, cleaned of excess fat, and weighed.

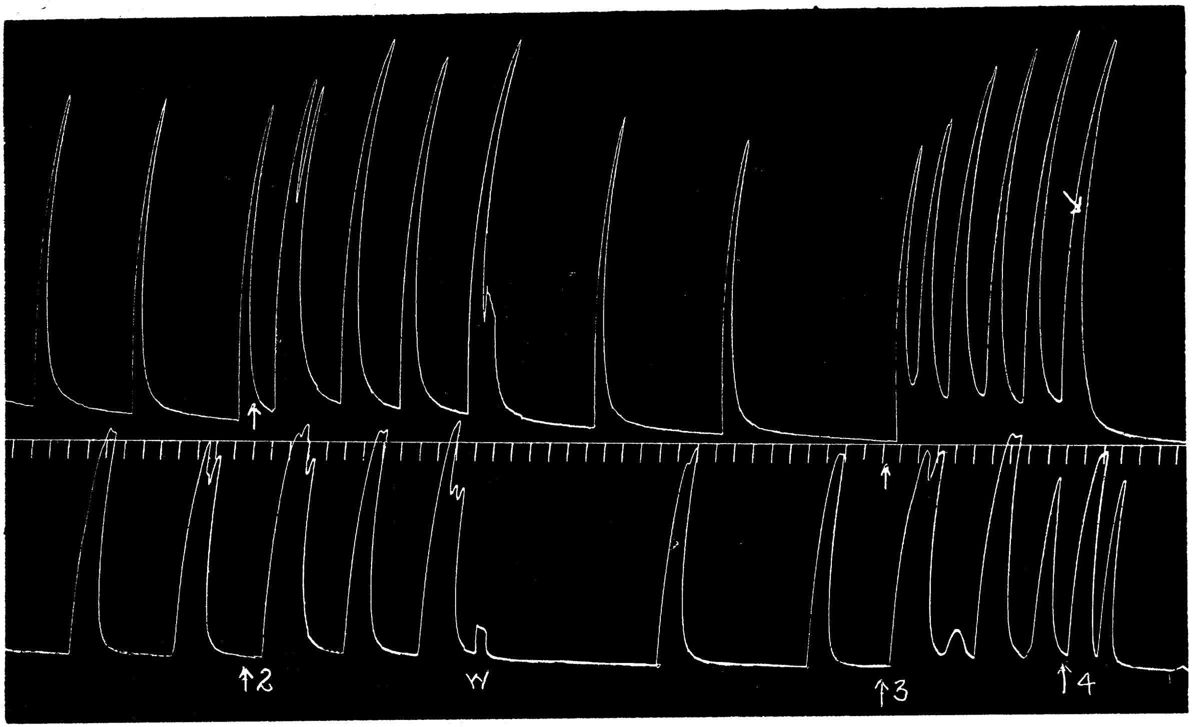

FIG. 1. Top curve shows the spontaneous contractions of the uterus taken from a rat on a choline-deficient diet (animal was deprived of yeast supplement during the last 14 days) and the bottom curve, the activity of a similar segment of uterus taken from a normal rat. Middle record is the time in intervals of 20 seconds. At the interval marked 2, acetylcholine chloride was added to the bath, making a 1 : 25,000,000 solution. At W, the acetylcholine-Locke's solution was replaced by fresh Locke's solution. At 3, posterior pituitary solution (1/250 U.S.P. unit) was added to the 100-cc bath. At 4, epinephrine was added to make a 1 : 2,000,000 dilution.

Segments of one horn of the uterus taken from each of two animals were placed in aerated Locke's solution at 38° C ± 0.2° for recording the contractions on a smoked drum surface. The method used was the same as that previously described by one of us (*5*). Generally, segments of one horn from an experimental animal and of one horn from a control animal were studied simultaneously. All preparations were tested for their response to posterior pituitary solution (1/5th to 1/1,000th unit in 100 cc bath), epinephrine solution (1:2,000,000) and acetylcholine (1: 25,000,000 to 1: 200,000,000).

During the course of the experiment, the fur of all nursing mothers and their litters became oily, apparently about the time the young started to get into the food containers in the cages, while at no time did oiliness of the fur appear in any nonlactating rat.

Table 1 reveals no essential differences in either the nursing or the nonlactating groups between the choline-deficient animals and their controls. There are, however, differences between the nursing animals considered as a group and the nonlactating rats as a group, indicative of an inadequacy of the diet for lactation, despite the addition of choline. Every nursing rat lost weight during the 39-day experimental period while every nonlactating rat gained weight. The uteri of the 6 nursing mothers varied in weight from 168 to 427 gm with an average of 268 gm, while the uteri of 10 nonlactating rats ranged from 318 to 560 gm with an average of 446 gm. The two nursing rats, one choline-deficient and one control, deprived of the yeast and casein supplement for the last 14 days of the experiment showed a marked loss in body and uterine weight, while the non-nursing postpartum choline-deficient rat on a similar regimen appeared entirely normal.

The vaginal smears showed regular four-day estrous cycles in both experimental and control nonlactating females, and continuous diestrous smears in all nursing rats, broken by one or two estrous periods near the end of the experiment.

All uteri, on being excised and placed in Locke's solution at room temperature, contracted spontaneously and remained contracted until placed in warm (38° C) aerated Locke's solution. Immediate spontaneous contractions were recorded from every uterus, with no significant differences in rhythmicity or force of the contractions (Fig. 1).

The rate of contraction and general tonus were increased by both acetylcholine and posterior pituitary solution, while epinephrine caused a decrease in the general tonus and usually cessation of the spontaneous activity in all uteri (Fig. 1). These effects were readily reversed by replacing the drug-Locke's solution by a fresh Locke's solution.

The animals in these experiments represent a rather wide range of choline ingestion from adequacy downward. Although the nine controls received 30 mg of choline every second day in their diet, the nursing mothers were deprived of a considerable and variable portion of this supplement during the last three weeks of the diet on account of the inroads of their young at the food containers. The experimental animals were obtaining small amounts of choline from the yeast supplement (*3*). The particular product used here provided 3.6 mg of choline every second. The two experimental animals, one nursing and one nonlactating, which were deprived of the yeast and casein supplement for the last two weeks of the diet, probably achieved a true choline-deficiency, but achieved as well a deficiency in protein, biotin, para-aminobenzoic acid, inositol and perhaps other B-complex factors. However, in this widely varied series of 16 rats, spontaneous contractility and normal reactivity to hormonal and humoral agents were observed in all uteri, irrespective of their source.

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A New Method of Inoculating the Maydeae with Smut Fungi

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Corn seeds soaked in sporidial suspensions of three smuts of the Maydeae showed smut galls soon after germination. This proved to be a new method of infecting corn seedlings with the corn smut organism. The infestation technique employed was the same as that described earlier by the author (*1*) in obtaining seed and seedling infection of the grasses and cereals with *Xanthomonas translucens*. This discovery added another method of inoculating corn with species of Ustilago. The well known method of promoting infection with *Ustilago zeae* is to bring the sporidia or chlamydospores in contact with the growing point down in the spiral whorl or to inject the spores hypodermically into the tissues of the host. Seed infestation with sporidial suspensions in water merely constituted a surface contamination of the seed under favorable conditions for the growth and development of the parasite and the host. Apparently the fungus gained entrance into the host through the germinating seed and eventually reached the crown of the young developing seedling. Infection occurred only when the seeds were infested with sporidia and not with chlamydospores. Some seedlings were killed immediately after emergence. Others developed large swellings or galls, and still others were not attacked. This method of infection was not limited to *Ustilago zeae*. Infection was

TABLE 1

THE RESPONSE OF SWEET AND DENT CORN INFESTED IN SPORIDIAL SUSPENSIONS OF THREE SPECIES OF SMUT FUNGI

Smut species	No. seeds infested	No. plants emerged	No. plants infected
		*Sweet corn**	
Ustilago zeae†	100	82	32
Ustilago dieteliana‡ on Tripsacum	260	210	81
Sphacelotheca reiliana from teosinte	360	322	0
		Dent corn§	
Ustilago zeae†	100	97	2
Ustilago dieteliana‡ on Tripsacum	420	400	29
Sphacelotheca reiliana from teosinte	360	348	0
		Teosinte‖	
Ustilago zeae†	40	25	0
Ustilago dieteliana‡ on Tripsacum	20	13	2
Sphacelotheca reiliana from teosinte	40	19	0

* Sweet corn variety Golden Bantam.

† *Ustilago zea* was collected near Ames, Iowa, and grown in single and mixed sporidial cultures for the infection trials.

‡ *Ustilago dieteliana* was collected by I. E. Melhus in 1945 near Mexico City, Mexico, on ***Tripsacum latifolium.*** Mixed sporidial cultures were used in the infection trials.

§ Dent corn commercial hybrid U. S..13.

‖ Teosinte obtained from field near Chalco, Mexico.

FIG. 1. ***Left***: healthy seedling; ***middle***: seedling galled by Tripsacum isolate; ***right***: seedling galled by corn isolate.

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