Table 1. Lesions in the reproductive tract of male mice exposed prenatally to DES. Males were the 9- to 10-month-old offspring of CD-1 mice treated with DES (100 μg/kg, subcutaneously) on days 9 to 16 of gestation.

Prenatal treatment	Incidence of lesions*	Location of lesions		
		Testis†	Epididymis‡	Accessory sex glands§
Corn oil	0/14	0	0	0
DES	18/24	15	10	6

*Expressed as the ratio: (number of male offspring with one or more reproductive tract lesions)/(number of male offspring examined). †Lesions included (i) intra-abdominal retention and/or fibrosis and calcification of testes, and (ii) reduction in number of spermatogonia with multinucleate cells in lumina of testes. ‡Epididymal cysts. §Lesions seen in the area of the seminal colliculus; the nodular masses of five animals were associated with squamous metaplasia; the cellular atypia in one animal resembled early neoplasia.

should be noted that testicular lesions may be secondary to cryptorchidism. However, the appearance of multinucleate giant cells within the seminiferous tubules of scrotal testes indicates an additional effect of prenatal DES treatment on the gonad.

Significant alterations were seen in the accessory sex glands of male mice exposed prenatally to DES (Table 1). These glands were usually distended with hard secretory material and spermatozoa. Nodular enlargements of the seminal vesicles or coagulating glands, or both, were found adjacent to the seminal colliculus of eight animals. Inflammation, including erosions or ulcers and escape of secretions into the surrounding tissues, was associated with enlargements of the seminal vesicle.

Nodular enlargements of the coagulating glands and ampullae found in five mice were associated with squamous metaplasia (Fig. 1c). Whether these metaplastic changes are preneoplastic or constitute benign lesions is unclear; squamous metaplasia of the accessory sex glands of male rodents given high doses of estrogen has been described (*3, 4*). However, adjacent to and in the duct of the coagulating gland of the prostate of one animal, there were downgrowths and cellular pleomorphism (Fig. 1d) suggesting a more serious, and possibly preneoplastic, growth disturbance.

Carcinoma of the male rodent accessory sex glands is very rare (*5*). Indeed, treatment of mice with large doses of DES as adults results in mammary (*6*) or testicular tumors (*7*), but no other genital tract neoplasms were noted. Similarly, neonatal administration of DES at a dose of approximately 1 g/kg failed to produce lesions of the male accessory sex glands although epididymal cysts and fibrotic testes were observed (*8*).

Another group of nine mothers was treated with DES in the present study. All of their 1- to 4-day-old male offspring had testes anterior to the urinary bladder or, in some cases, firmly fixed to the posterior pole of the kidney. In the young from a corresponding number of control mice, the testes were always at the level of the bladder. Similarly, Greene *et al.* (*4*) found intra-abdominal testes in newborn rats exposed prenatally to high doses of estradiol diproprionate.

In light of these results in rodents, the incidence of cryptorchidism in young boys whose mothers had been treated with DES during gestation may be of clinical importance. Obviously, these offspring should be further evaluated for latent alterations of the genital tract, since changes in the adult male human reproductive tract similar to those we observed in the mouse might be dismissed as secondary to inflammation. Some of these lesions could be important causes of infertility even when viable sperm are produced.

The observed effects of DES on the reproductive tract may be explained, in part, by considering the development of this organ system. In the female mouse (*9*) and human (*10*), the Müllerian ducts are the fetal precursors of the definitive oviduct, uterus, and anterior portion of the vagina. It has been suggested that the embryonic origin of the DES-induced vaginal adenocarcinoma in women is Müllerian duct tissue (*11*); these suggestions are reinforced by studies with DES on the vaginal epithelium of neonatal (*9, 12*) and fetal mice (*13*). Although the Müllerian ducts regress during the normal differentiation of the genital tract in the male, vestiges of these embryonic tissues do persist as the appendix testis and prostatic utricle (*10, 14*). The positions of the fibromuscular growths and nodular masses in the male offspring of DES-treated mice are consistent with the anatomical locations of the appendix testis and prostatic utricle, respectively. Although an action of DES on the fetal urogenital sinus must be considered, these findings raise the possibility that the disturbance of genital tract development following prenatal exposure to DES may be mediated, in part, through Müllerian tissue in the male, as well as the female, fetus.

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Lysinoalanine: Presence in Foods and Food Ingredients

Abstract. *Lysinoalanine, N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine, an unusual amino acid implicated as a renal toxic factor in rats, has been found in proteins of home-cooked and commercial foods and ingredients. Although it has been reported to occur in both edible and nonfood proteins only after alkali treatment, it has now been identified in food proteins that had not been subjected to alkali. Lysinoalanine is generated in a variety of proteins when heated under nonalkaline conditions.*

The preference of affluent industrialized societies for manufactured foods as opposed to home cooking has prompted consideration of the need to determine the effect of processing procedures on the wholesomeness and nutritive value of various foods. Heating and alkali treatment have been reported to adversely affect the nutritional value of proteins (*1, 2*). Heating may abolish the nutritive value of essential amino acids originally present in a protein by means of racemization (*3*) and chemical

decomposition (*4*). Alkali treatment of proteins above a *p*H of 10.5 at 25°C or above a *p*H of 8 in boiling water causes the formation from lysyl and cystinyl or glycosidically bound seryl residues of a new amino acid, N^{ϵ}-(DL-2-amino-2-carboxyethyl)-L-lysine (*5*), trivially called lysinoalanine (LAL).

The presence of LAL in the structure of proteins has been reported (*2*) to reduce protein digestibility and net protein utilization. There have been reports of renal lesions characterized by cytoplasmic and nuclear enlargement of the tubular epithelium in the pars recta (so-called nephrocytomegalia) in rats fed alfa protein, a modified soya protein used for industrial purposes, and soya protein subjected in the laboratory to severe alkali treatment in 0.1*N* NaOH at 60°C for 8 hours (*6*). Other workers have been unable to confirm nephrocytomegalia in rats fed spun soy isolate, which is an alkali-treated edible soy protein (*7*). However, when rats were fed either synthetic LAL or soya protein that had been alkali-treated and subsequently acid-hydrolyzed (in other words, LAL was not a residue within the structure of a protein but was fed as a free amino acid), marked nephrocytomegalia accompanied by tubular nephrosis, necrosis, and regeneration of epithelial cells were noted (*8*). These changes seemed, however, to be species-specific to the rat. Mice, hamsters, Japanese quail, dogs, and monkeys failed to exhibit renal cytomegalic effects when fed synthetic LAL (*9*).

At present there are a number of food processes in which alkali treatment is used with the potential to generate LAL residues in the respective food proteins. Examples of foods that undergo alkali treatment in the manufacturing process are hominy, pretzels, and sausage casings. During storage of chicken eggs the *p*H of the egg white gradually increases up to 9.0. Alkali cooking of maize, a staple cereal of people in Central America who subsist on tortillas, is a traditional processing technique in the New World, used to soften the pericarp and increase the digestibility (*10*).

In view of the above considerations we were interested to learn the extent to which LAL occurs in foods. The results of LAL assays in food ingredients, home-cooked foods, and commercial food preparations are presented in Table 1. Samples of ingredients were obtained from industrial suppliers and commercial foods purchased from food stores, and home-cooked foods were prepared in the laboratory from groceries. Moist materials were freeze-dried, and those containing fats were extracted at room temperature with a 2:1 mixture of chloroform-ethanol.

Samples were hydrolyzed for 4 hours in

Table 1. Results of LAL assays in home-cooked foods, food ingredients, and commercial food preparations.

Name	Origin	LAL (micrograms per gram of protein)
	Home-cooked foods	
Frankfurter	As purchased, before heating	None
Frankfurter	Boiled	50
Frankfurter	Fried	50
Frankfurter	Oven-baked	170
Frankfurter	Charcoal-broiled	150
Chicken thigh	Raw	None
Chicken thigh	Charcoal-broiled	150
Chicken thigh	Retorted	100
Chicken thigh	Cooked in microwave oven	200
Chicken thigh	Oven-baked	110
Chicken thigh	Retorted in gravy	170
Pan scrapings	Pan-frying of sirloin steak	130
Egg white	Fresh	None
Egg white	Boiled 3 minutes	140
Egg white	Boiled 10 minutes	270
Egg white	Boiled 30 minutes	370
Egg white	Pan-fried 10 minutes at 150°C	350
Egg white	Pan-fried 30 minutes at 150°C	1,100
	Commercial food preparations	
Corn chips	Commercial sample	390
Pretzels	Commercial sample	500
Hominy	Commercial sample	560
Tortillas	Commercial sample	200
Taco shells	Commercial sample	170
Milk, infant formula	Commercial sample, manufacturer A, batch 1	330
Milk, infant formula	Commercial sample, manufacturer B, batch 1	550
Milk, infant formula	Commercial sample, manufacturer A, batch 2	150
Milk, infant formula	Commercial sample, manufacturer A, batch 3	640
Milk, infant formula	Commercial sample, manufacturer B, batch 2	510
Milk, infant formula	Commercial sample, manufacturer C	490
Milk, evaporated	Commercial sample, manufacturer D	860
Milk, evaporated	Commercial sample, manufacturer E	590
Milk, skim, evaporated	Commercial sample, manufacturer D	520
Milk, condensed	Commercial sample, manufacturer F	540
Milk, condensed	Commercial sample, manufacturer G	360
Simulated cheese	Commercial sample, manufacturer H	1,070
	Food ingredients	
Egg white solids, dried	Commercial sample, manufacturer I	1,820
Egg white solids, dried	Commercial sample, manufacturer J	1,530
Egg white solids, dried	Commercial sample, manufacturer K	490
Egg white solids, dried	Commercial sample, manufacturer L	160
Calcium caseinate*	Commercial sample, supplier M	1,000
Calcium caseinate	Commercial sample, supplier N	370
Sodium caseinate	Commercial sample, supplier O	600
Sodium caseinate	Commercial sample, supplier P	6,900
Sodium caseinate	Commercial sample, supplier Q	1,190
Sodium caseinate	Commercial sample, supplier R	430
Sodium caseinate	Commercial sample, supplier S	800
Acid casein	Commercial sample, supplier T	140
Acid casein	Commercial sample, supplier U	190
Acid casein	Commercial sample, supplier V	70
Masa harina	Commercial sample	480
Hydrolyzed vegetable protein	A total of 18 commercial samples of different batches from five manufacturers	40–500
Whipping agent	Commercial sample, manufacturer's type 1	6,500
Whipping agent	Commercial sample, manufacturer's type 2	50,000
Soya protein isolate	A total of 45 commercial samples of different batches from two manufacturers	0–370
Yeast extract	Commercial sample	120

*The identity of the manufacturer was not determined; therefore, the term supplier was used instead.

Table 2. Formation of LAL in proteins by heating under nonalkaline conditions.

Protein	Concentration (% by volume)	Temperature (°C)	Time (hours)	*p*H	LAL (micrograms per gram of protein)
Bovine serum albumin	1	120	1	6.0	3500
Casein	1	120	1	6.0	1700
Lysozyme	1	120	1	2.0	None
Lysozyme	1	120	1	4.0	275
Lysozyme	1	120	1	6.0	1000
Ovalbumin	1	120	1	2.0	150
Ovalbumin	1	120	1	3.0	250
Ovalbumin	1	120	1	4.6	310
Ovalbumin	1	120	1	6.0	570
Soya globulin	6	120	16	2	<40
Soya globulin	6	120	16	3	<40
Soya globulin	6	120	16	4	80
Soya globulin	6	120	16	5	120
Soya globulin	6	120	16	6	130
Soya globulin	6	120	16	7	180
Soya globulin	6	100	1	6.5	<40
Soya globulin	6	100	2	6.5	80
Soya globulin	6	100	3	6.5	130

air-evacuated sealed ampuls with 6*N* HCl at 120°C for determinations by ion-exchange chromatography with an automated amino acid analyzer (Technicon) (*2*) and for 24 hours at 110°C for determinations by thin-layer chromatography (TLC) (*11*).

Most samples were analyzed by both methods except those that gave an interference with the LAL peak on the ion-exchange chromatographs. The identity of LAL determined only by TLC was confirmed by preparative separation on paper chromatography with the TLC solvent system, elution of the LAL area with water, and determination of LAL by ion-exchange chromatography.

The results of the determinations (Table 1) reveal the widespread occurrence of LAL in home-cooked and commercial foods and ingredients that had not been exposed to alkali. Particularly significant is the finding of LAL in condensed milk, acid casein, cooked chicken thigh, and sirloin steak pan scrapings, none of which were exposed at any time to an alkaline medium. Therefore, we hypothesize that LAL does form in proteins under conditions that do not entail alkali treatment.

Soya globulin, ovalbumin, lysozyme, casein, and bovine serum albumin (*12*), all proteins occurring in food systems, were heated under nonalkaline conditions. All formed variable amounts of LAL in the range of *p*H, temperature, and time commonly used in home cooking and commercial processing (Table 2).

In light of the experiments connecting the presence of LAL in proteins with reduced nutritional availability and possible toxic effects, our finding of LAL in the structure of heated proteins not necessarily subjected to alkali treatment may be a factor to be considered in explaining the reduction of nutritional value by heating. The formation of LAL in proteins by heating at *p*H values considerably lower than those obtained during alkali treatment, as reported in (*5*), suggests the ubiquity of LAL in cooked foods and that humans have long been exposed to proteins containing LAL.

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Stage-Specific Switches in Histone Synthesis During Embryogenesis of the Sea Urchin

Abstract. *Histones H2A and H2B of the sea urchin embryo have been resolved by new methods into components that are synthesized at different stages of development. One form of H2A and one form of H2B are synthesized only during the period from fertilization to the blastula stage. Subsequently, two other types of H2A and H2B molecules are synthesized. In addition, a histonelike protein was detected which is synthesized only from fertilization until the 16-cell stage when the synthesis of still another H2A-like protein begins. None of the late-appearing forms are derived from histone polypeptide chains synthesized earlier in development. Since the early components do not disappear after their synthesis stops, these modulations of histone synthesis lead to an increase in histone multiplicity, concomitant with the beginning of cell diversification and a decrease in cell division rate.*

Histones are the principal proteins of eukaryotic chromosomes. Although they have many properties that make them suited for long-term gene regulation (*1*), the failure of conventional methods to demonstrate extensive histone diversity or tissue-specific variability has discouraged serious consideration of a regulatory role for these proteins. However, over the last few years one of the five major histone types, H1 (*2*), has been shown through the application of improved methods to be a family of related protein components that exhibit considerable tissue-specific variation (*3*). In the sea urchin the complexity of H1 histones increases from a single species in morulae to three species in gastrulae (*4, 5*).

Histones H2A, H2B, and H3 are difficult to resolve by standard electrophoretic techniques because of their similarity in molecular size and ionic charge. Their resolution can be improved by performing the