purination; this in turn causes scission of the phosphodiester backbone[7] leading to lower sedimentation coefficients. Heating of the modified DNA results 1. in further depurination and hence further scission and 2. in separation of the strands and therefore the unmasking of additional 'hidden' (single-chain) breaks that previously were stabilized (i. e. hidden) by the double-stranded structure. These effects cause a further reduction in sedimentation coefficient (Table). The control DNA, on the other hand, does not show this pronounced decrease in sedimentation coefficient upon thermal denaturation.

Additional experiments revealed that exposure of deoxyguanosine to these substances resulted in the formation of a product with chromatographic and spectral properties similar to those of 7-methyldeoxyguanosine. These findings support the above postulated mechanism of action in which alkylation of DNA is the first step in the degradative process, they are also in accord with the known alkylating potential of these substances (see refs.[1] and[5]).

In view of the established relationship between the ability of a substance to react with DNA and its potential to induce detrimental effects (mutations, carcinogenesis and teratogenesis) and because of widespread human exposure to the agents studied, ways to eliminate human contact with these substances should be sought[8].

Effects of phosphoric acid esters on the properties of DNA

Additions	Sedimentation coefficients (s) Before heating	After heating
None	18.4	17.5
Dipterex	11.2	7.4
DDVP	15.7	9.1
Trimethylphosphate	15.7	10.3

To 1 ml of calf thymus DNA (1 mg per ml of 0.015 *M* NaCl in 0.01 *M* phosphate buffer, pH 7.0) either 1 µl of DDVP, 1 µl of trimethylphosphate or 100 µg dipterex were added. The mixtures were incubated at 56°C for 42 h whereupon DNA was precipitated with ethanol, the insoluble fibers washed extensively with ethanol and the DNA redissolved in 0.15 *M* NaCl. Portions of the specimens were placed in a boiling water bath for 10 min. and then immersed into an ice-bath. Sedimentation coefficients were determined in a Spinco Model E analytical ultracentifuge equipped with an ultraviolet optical system.

Résumé. L'addition de phosphate triméthyl, «dichlorvos» (0, 0-dimethyl-2, 2, dichlorovinyl phosphate) et du «diptérex» (0, 0-dimethyl-2, 2, 2-trichloro-1-hydroxyethyl phosphonate) à du DNA provoque la dégradation de cette macromolécule. La réaction semble être due à une alkylation du résidu de guanine du DNA. Ces observations peuvent fournir une explication chimique aux effets biologiques de ces substances.

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15 November 1972.

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Fluorescence of the Soluble 'Sweet-Sensitive' Protein Complexes with Sugars

The interaction of a 'sweet-sensitive' protein with sugars and saccharin was originally studied by the method of difference spectroscopy[1]. Changes in optical density upon titration with various concentrations of different sugars were recorded at 277 nm, the ultraviolet maximum of the protein using a tandem double compartement technique[2]. The UV-absorption spectra of proteins is mainly due to their content of the aromatic amino acid tyrosine, tryptophan and, to a lesser extent, phenylalanine[3]. The region 278–281 nm is characteristically the maximum peak area[4].

The fluorescence of proteins originates almost entirely from the tyrosyl and tryptophenyl residues. Consequently, conformational modifications can be followed by observing changes in either tyrosine or tryptophan fluorescence intensity. This study of the fluorescence of the 'sweet-sensitive' protein and its sugar complexes was undertaken because of the known sensitivity of the emission of the aromatic chromophores.

Materials and methods. The 'sweet-sensitive' protein was extracted from epithelium of cow and dog tongues by the method previously described[1,5] with the following exceptions: 1. the 75,000 × *g* or 105,000 × *g* supernatant was not subjected to ammonium sulfate but was fractionated by the ultrafiltration in an Amicon cell with a Diaflow membrane of retention of molecular weights 100,000 and up: 2. the retentate from ultrafiltration was subjected to cation exchange on Bio-Rex 63 resin (control No. 7429) and the protein eluted with 1.0 *M* sodium bicarbonate-sodium carbonate buffer pH 10.0 plus 1 *M* NaCl. The protein was dialyzed in the ultrafiltration cell until equilibrium to 0.1 *N* sodium phosphate buffer pH 7.0 was achieved.

Emission spectra were measured with an instrument built in the laboratory[6]. Optical density (OD) recordings were made on a Cary 15 recording spectrophotometer. In

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general, all solutions had an OD less than 0.10. Relative quantum yields at 25 °C were calculated by the relation:

$$Q^7 = \frac{\text{Emission Area standard}}{\text{Emission Area sample}} \times \frac{\text{OD standard}}{\text{OD sample}} \; 0.20^8 \quad \text{or} \quad 0.21$$

The protein from dog tongues (D-13) as well as from cow tongues was excited at 270, 280 and 295 nm and its emission spectra recorded. The quantum yield was greatest at excitation of 280 nm with a peak maximum at 330. An experiment consisted of dispensing 2 ml of diluted protein in 0.1 M sodium phosphate buffer pH 7.1 into 2 separate cuvettes recording the emission spectra and then the OD. Next, one of the cuvettes would be titrated with sugar in a buffer and the other an equivalent amount of buffer only[7].

Results and discussion. The emission spectra of the protein from dog tongues and the complex with 0.095 M glucose is shown in Figure 1. The results of the cow protein studies were similar to that of the dog. The respective areas correspond to the relative quantum yields. The addition of glucose results in a decrease of the quantum yield of the protein or a quenching effect. There does not appear to be a shift in the maximum of the complexed protein. A range of glucose concentrations from 0 to 0.75 M was next studied to determine the extent of quenching with concentration. The results of this study are seen in Figure 2. The quantum yield of the complexed protein decreases from 0 to 0.33 M, increases slightly to 0.57 M, then decreases to 0.75 M. The effect of increasing concentrations of glucose appears to result in a sigmoidal quenching of the fluorescence. The native protein in Figure 2 is excited at 280 nm and diluted with buffer in equal volumes as the experimental but without glucose. This control is necessary to take into account any changes in the quantum yield due to irradiation and dilution of the protein.

Sucrose was then examined for the ability to modify the quantum yield of the protein and the results of this experiment are presented in Figure 3. Unlike glucose, the effect of sucrose on the fluorescence of the protein is one of exhaltation or increase of the quantum yield. The magnitude of change from the control is also greater, but again a sigmoidal pattern of change is noted. There is an increase from 0 to 0.184 M, then a drop to 0.33, followed by a steep rise to 0.57 M sucrose. The effect of sucrose on the

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[8] Quantum efficiency of tryptophan and tyrosine, respectively.

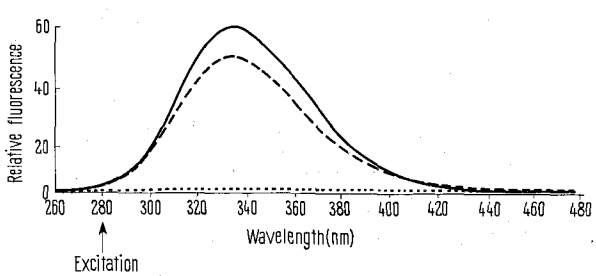

Fig. 1. Fluorescence spectra of dog 'sweet-sensitive' protein and the protein and glucose complex. Conditions: pH 7.1, 0.1 M sodium phosphate buffer, 25°C. —, protein; ---, protein plus 0.095M glucose; ....., 0.095M glucose in buffer.

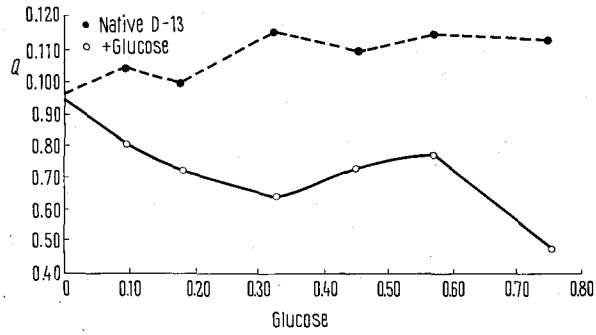

Fig. 2. Protein quantum yields of glucose complexed and free (native) dog 'sweet-sensitive' protein. Native or free protein was excited at 280 nm the same number of times as the complexed protein, but with dilutions of buffer instead of glucose in buffer.

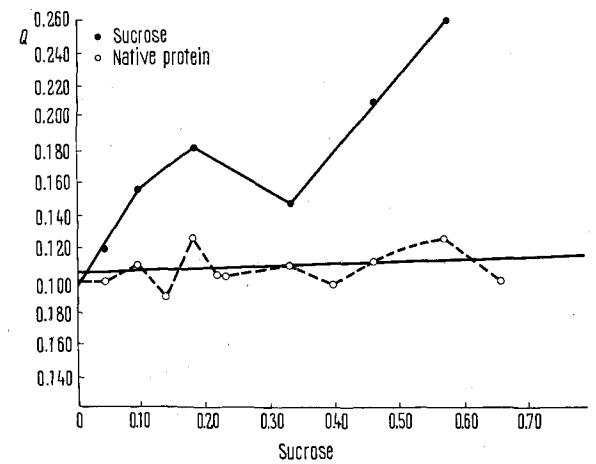

Fig. 3. Protein quantum yields of complexes of dog 'sweet-sensitive' protein with sucrose. Native or control was the same protein, excited at 280 nm the same number of times as the complexed but with dilutions of buffer instead of sucrose in buffer.

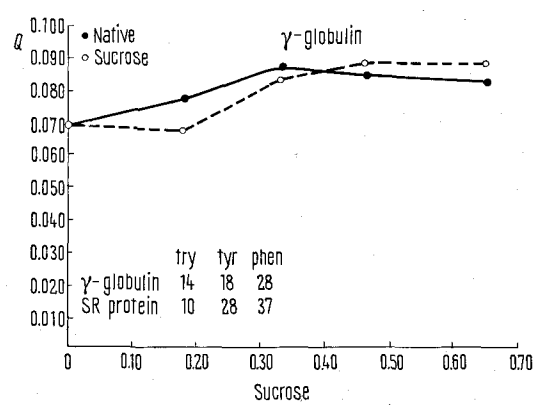

Fig. 4. Protein quantum yields of γ-globulin w/wo sucrose. Sucrose curve is protein titrated with sucrose in buffer at concentrations indicated and quantum yields determined. Native protein was titrated with buffer – 0.1 M sodium phosphate pH 7.0 in same volumes as sucrose curve. SR, soluble receptor 'sweet-sensitive' protein from the dog. Try., tyr., and phen. tryptophan, tyrosine, and phenylalanine, respectively.

fluorescence pattern of the 'sweet-sensitive' protein then is quite dissimilar to that of glucose. While the greater magnitude of change noted with sucrose versus glucose might be due to the greater sweetness of sucrose in the dog[9], the enhancement by sucrose and the quenching by glucose is not so easily explained. One readily apparent possibility is that there are separate sites for interaction by monosaccharides (glucose) and disaccharides (sucrose), and that these interactions result in quenching and exhaltation, respectively. Studies involving different monosaccharides and disaccharides are in progress.

To ascertain the ability of the sugars to non-specifically affect the quantum yield values of a protein, a control was examined. Bovine γ-globulin of similar molecular weight and aromatic amino acid content[10], as the dog (unpublished amino acid analysis), was subjected to glucose and sucrose titration under identical conditions. The results of the sucrose study are presented in Figure 4. The protein titrated with either sucrose, glucose, or buffer gave similar fluorescence quantum yields. No quenching or exhaltation was observed.

The interactions of sucrose and glucose with the dog 'sweet-sensitive' protein appear to be specific. The fluorescence technique would seem to be ideally suited for study of those interactions because of the high sensitivity of emission of the aromatic chromophores[11].

Zusammenfassung. Komplexe, welche das «süssigkeitsempfindliche» Protein mit Glukose und Rohrzucker bilden, wurden mit Hilfe der Fluoreszenz-Spektroskopie untersucht.

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U.S. Natick Laboratories, Pioneering Research Laboratory, Natick (Massachusetts 01760, USA), 25 October 1971.

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[11] Acknowledgment. The advice, suggestions and technical assistance of Mr. LEO G. HOLMES are greatly appreciated. The study was supported by an Associateship from the National Academy of Sciences-National Research Council and a grant from the Army Research Office.

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In vitro and in vivo Catabolism of Cholesterol in Meal-Fed Rats

A decrease in feeding frequency in chickens[1,2] and rabbits[3] potentiates distinctly the effect of atherogenic cholesterol diet. In meal-fed monkeys, the level of serum cholesterol is higher also when these are fed a diet without cholesterol[4]. Similar experiments with rats did not give explicit results: only in females fed a cholesterol diet the meal-feeding regimen did increase cholestrolemia[5]. On the contrary, we have found in our experiments that the application of a long term meal-feeding regimen in rats has caused a cholesterol accumulation in some organs and in the carcass of the experimental animals also when a diet without cholesterol was used. Therefore in this work we have followed the extent of cholesterol catabolism to bile acids as one of the possibilities through which the meal-feeding regimen interferes in the regulation of cholesterol metabolism. There are no experimental data on this problem up to now.

Experimental. In all experiments male Wistar rats initially weighing about 220 g, fed a standard diet[6] were used. The control animals were fed ad libitum; access to food of the animals from the experimental group was gradually shortened in the course of 8 weeks down to 2 h daily (in the first 2 weeks 8 h daily, in the 3rd and 4th week 6 h, in the 5th and 6th week 4 h, in the 7th and 8th week 3 h daily). During the following 35 weeks, the meal-fed group was fed 2 h daily between 08.00–10.00 o'clock. All animals had free access to water. In all cases the animals were decapitated at the end of the experiment after 22 h of starvation. In extracts[7] of blood serum, some tissues and carcass[8], total cholesterol was determined[9].

In vitro experiment. At the end of the experiment, immediately after decapitation, liver was quickly excized

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Table I. Cholesterol level in blood serum and organs of ad libitum and meal-fed rats

Group	Serum	Liver	Small intestine	Adrenals	Epididymal fat	Brain	Carcass
No. of animals	10/15[a]	10/15	10/15	9/13	10/14	10/15	10/8
Ad libitum	55 ± 5[b]	273 ± 9	208 ± 11	2096 ± 183	35 ± 4	1355 ± 88	112 ± 8
Meal-fed	57 ± 4	325 ± 27	288 ± 17	3260 ± 277	103 ± 8	1371 ± 45	147 ± 12
Statistical significance (P)	—	—	< 0.002	< 0.01	< 0.001	—	< 0.05

[a] Number of animals in ad libitum fed group/in meal-fed group. [b] Mean values ± S.E. The values are expressed in terms of mg per 100 ml of blood serum or 100 g of wet wt. of tissue, in the case of carcass per 100 g of dead weight of the animal without gastrointestinal tract.