The established role of carbonic anhydrase[4] in fluid and ion exchange processes tempts one to speculate on a possible relation between the presence of this enzyme in the ubiquitous microbial inhabitants of the naso-pharynx and the symptoms of the common cold. Two possibilities immediately present themselves. (1) Carbonic anhydrase is present in these organisms and plays a part in the excretion of certain products of microbial metabolism: that is, acids, and/or the ill-defined slimes known to be produced[5]. These excretory products could in turn irritate the mucous membranes of the naso-pharynx altering their permeability and susceptibility to invasion. (2) The presence of extra-cellular carbonic anhydrase could disturb the normal excretory mechanism of the mucous membrane tissue cells themselves, leading to changes in permeability and resistance to invasion. We find these two postulated mechanisms intriguing and intend further studies in this area.

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Unusual Behaviour of a Mammalian Collagen in Deuterium Oxide

Gross[1] observed that heating a solution of collagen in phosphate buffer to 37° C resulted in the formation of an opaque gel of collagen fibres. That this occurred at 37° C, but not in the cold, suggests that some unfolding of the collagen molecule is required to form fibres or gels, and that newly synthesized collagen molecules, *in vivo*, are also not as highly ordered as possible, but are somewhat unfolded in order to form connective tissue fibres. To test this idea *in vitro* we proposed to conduct the Gross heat gelation in deuterium oxide. Harrington and von Hippel[2] showed that in the case of ichthyocol collagen in deuterium oxide, the helix→coil transition occurs at a temperature 4° C higher than in water. If heat gelation of collagen in deuterium oxide were to require a higher temperature than in water, the hypothesis would be supported. When we attempted to carry out the experiment, unexpected results were obtained, of which a preliminary report is presented here.

Soluble collagen was obtained from rabbit skin by extraction at 5° C with two changes of 0·5 M sodium acetate, followed by citrate buffer of *p*H 3·5. The citrate solution was dialysed against *p*H 7·6 phosphate buffer and distilled water to yield collagen fibres which were collected by centrifugation, washed three times with distilled water and freeze-dried for storage.

Solutions of collagen were made and treated as shown in Table 1. Electron micrographs of the fibres *D* showed them to be about 500 Å wide and unstructured. It is of interest that after dialysis against water–phosphate fibres did not form on dialysis against deuterium oxide–phosphate (*C*), although in the original preparation the collagen had been dialysed against aqueous phosphate, although of a different strength and *p*H.

When *B* was slowly warmed the opaque gel of Gross[1] began to form at 35° C, and finally disappeared at 52° to give a clear gelatin solution. When *C* was heated, the opaque gel began to form at 25° C and finally disappeared at 56°. The range of existence of the deuterium oxide gel is thus 14° larger than that of the water gel, a remarkably large protein deuterium effect. Solutions *B* and *C* gave single hypersharp peaks in the ultracentrifuge.

Table 1. Behaviour of Collagen in Deuterium Oxide and Water Buffers (0·04 M Sodium Dihydrogen Phosphate, 0·09 M Disodium Hydrogen Phosphate, *p*H 7·2) at 5° C

0·2 per cent collagen in 0·5 M acetic acid in water or deuterium oxide, solution *A*

A dialysed against water-phosphate buffer to yield clear, viscous solution *B*	*A* dialysed against deuterium oxide–phosphate buffer to yield fibres, *D*. Larger yield of fibres when *A* is made in deuterium oxide
B dialysed against deuterium oxide–phosphate to yield clear, viscous solution *C*	

When collagen in aqueous citrate buffer (0·1 M sodium citrate, *p*H 4·3) was heated, it merely became converted to gelatin on reaching about 40° C, as indicated by a large decrease in viscosity. However, in deuterium oxide–citrate the solution became turbid at 25° C, then opaque at about 29° C and deposited fibres.

Possible slight differences of acidity between the water and deuterium oxide solutions have been ignored so far. Both heat gelation[1] and denaturation in acid citrate[3] are known to be insensitive to such small *p*H differences. After the initial observations were made, we carried out the next experiments with deuterium oxide double-distilled from permanganate; identical results were obtained. Nevertheless, we are using purified deuterium oxide in all the present work.

We are carrying on an extensive investigation of the chemical and physical properties of various collagens in deuterium oxide, and of fibres conditioned with deuterium oxide. This solvent is also being used to extract collagen and other macromolecules from biological sources.

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Cholesterol Esters of the Cockroach *Eurycotis floridana*

Investigations of the metabolism and occurrence of sterols in insects have led to several reports of the presence of sterol esters in these organisms[1–3], and some observations concerning the characteristics of a sterol esterase in at least one species of cockroach have been recorded[1]. Up to the present time, however, no work on the constitution of the sterol esters of an insect has been described. We have undertaken such a study in relation to a broader investigation of the functions of sterols in the cockroach, *Eurycotis floridana*, and some of our preliminary findings are reported here.

The cockroaches used in this work were reared aseptically by the technique which has been described[4]. Materials from insects reared on three diets of distinctly different fatty acid content were analysed. Diet 1 was that which is used routinely in our laboratory and consisted of the semi-synthetic diet of Noland and Baumann[5], in which the corn oil was replaced by commercial sodium oleate. Diet 2 was identical with diet 1, except that the corn oil was replaced by a mixture of saturated fatty acids. Diet 3 was a commercially available dog food. The individual fatty acid components of these diets are given in Table 1 as percentages of the total fatty acid present.