Comments and Communications

Reaction of Bromine Water with Alfalfa Saponins

The recent report that alfalfa saponins are involved in bloat of ruminants has created interest in this group of compounds (*1*). Investigations in our laboratory have been centered on a mixture of saponins isolated from alfalfa by Walter *et al.* (*2*). No pure glycosidic compound has been isolated from this mixture, and paper chromatographic techniques have failed to resolve it into its component parts.

During a cursory examination of the effect of various oxidizing agents on alfalfa saponins we observed that saturated bromine water reacts rapidly with this mixture at room temperature, as evidenced by the disappearance of the bromine color, by the formation of a white precipitate, and by paper chromatographic studies.

Examination of the filtrate from the bromine water treatment by paper chromatography (*3*) revealed three spots on the chromatogram, all of which moved more slowly than the control sugars glucose, arabinose, xylose, and rhamnose. The rate of movement of the three spots suggested an oligosaccharide type of material and the spots gave the characteristic pentose-color reaction with the aniline spray reagent.

When untreated alfalfa saponins are hydrolyzed for two hours with 1*N* hydrochloric acid in a sealed tube in a boiling water bath, paper chromatography always reveals glucose, arabinose, xylose, and rhamnose on the chromatogram. When each of the three carbohydrate fractions obtained from the bromine-treated saponin was hydrolyzed similarly with 1*N* hydrochloric acid, the two slowest fractions were each composed of arabinose, xylose, and rhamnose. These two fractions contain the same sugars, but preliminary studies have indicated that the molecular ratios of the component sugars may be different. The third and fastest fraction consisted of glucose, arabinose, xylose, and rhamnose. The experimental data thus indicate that the bromine water cleaves sugars from the alfalfa saponins in the form of three sugar polymers. It seems appropriate to report these findings at this time because of Potter and Kummerow's (*4*) recent report on the isolation of three triterpene genins from dehydrated alfalfa leaf meal.

ARTHUR BEVENUE
KENNETH T. WILLIAMS

Agricultural Research Service,
U.S. Department of Agriculture,
Albany 6, California

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Free Amino Groups of Human Serum Albumin

It has been reported by Brand and Van Vunakis (*1*), using Sanger's DNP technique (*2*), and it is confirmed in this paper that there is one free alpha amino group, belonging to aspartic acid, in human serum albumin. The same free amino group was also found in bovine serum albumin by Brand and Van Vunakis.

McClure, Schieler, and Dunn (*3*), using Edman's phenyl isothiocyanate technique with bovine albumin, however, found a number of terminal alpha amino groups, of which two or three were contributed by aspartic acid, one by methionine, one by histidine, and an undetermined number by alanine.

Weber has observed an increased availability of the terminal groups of bovine serum albumin in methyl ethyl ketone. This is demonstrated by an increased rotational freedom of groups in the molecule, presumably about many bonds (*4*). It was postulated that the increased rotational freedom may make accessible to Sanger's fluorodinitrobenzene reagent groups that would otherwise be masked, and that these additional groups might account for the other free alpha amino groups found by McClure *et al.*, but not found by Brand and Van Vunakis.

It was of interest to know whether this might be true for human serum albumin. The DNP reaction was carried out first in the usual way as described by Sanger (*2*) by adding 1 g of 1:2:4 fluorodinitrobenzene (FDNB) in 10 ml of 95-percent ethyl alcohol to 0.5 g of human serum albumin dissolved in 5 ml of 10-percent sodium bicarbonate, *p*H 8.5. The mixture was shaken for 2 hr. The insoluble DPN albumin was washed three times each with water, alcohol, and ether, and then it was air-dried. A second sample was prepared in the same manner except for the fact that the FDNB was dissolved in 10 ml of methyl ethyl ketone, and the albumin was dissolved in 5 ml of a 10-percent solution of sodium carbonate, sodium bicarbonate, *p*H 9, the *p*H at which McClure's group worked. One hundred milligrams of air-dried DNP albumin were found by Thompson (*5*) to contain 79 mg of moisture-free albumin by amide determinations, and these figures were used here. Fifty-milligram samples of each preparation were hydrolyzed in 5.7*N* HCl by boiling under reflux for 4 hr and for 24 hr. DNP amino acids were identified by silica gel and filter paper chromatography (*2, 6*), the silica gel chromatography being quantitative. Control experiments showed that there was a 65-percent breakdown of DNP aspartic acid during 24 hr of hydrolysis. The 15-percent breakdown in 24 hr for the epsilon DNP lysine used was that reported by Sanger (*2*).

The results were the same in aqueous methyl ethyl ketone as in aqueous ethanol: one alpha amino group of aspartic acid (0.365 and 0.345 after 24-hr hydrolysis, corrected to 1.04 and 0.98, respectively) and

an average of 57 epsilon amino groups of lysine (56 and 59, respectively) per molecule of human serum albumin, molecular weight 69,000. There was no apparent freeing of additional alpha amino groups of human serum albumin detectable by this method.

HENRY BROWN

Department of Surgery,
University of Wisconsin, Madison

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Importance of Proteases as Factors Involved in the Exsheathing Mechanism of Infective Nematode Larvae of Sheep

The importance of pepsin as a factor influencing the exsheathment of third-stage infective nematode larvae in the host has been raised by Crofton (*1*). Working with *Trichostrongylus retortaeformis* from the rabbit, he obtained a high rate of exsheathment by using solutions of pepsin in hydrochloric acid. Solutions of hydrochloric acid alone and of distilled water gave no exsheathment, and boiling the pepsin solutions rendered them inactive.

The researches of Poynter (*2*), however, on infective nematode larvae of parasites of the horse indicate that enzymes do not have a role in the exsheathing mechanism. Sommerville (*3*) recently obtained rapid exsheathment *in vivo* with several species of infective larvae of sheep by using cellophane dialysis membranes in fistulas of the rumen and abomasum. Exsheathment has similarly been obtained in this laboratory at a slower rate and using an abomasal fistula only.

Further evidence that enzymes do not play a part in the exsheathing mechanism of infective larvae of sheep has been obtained by studying the action of pepsin and trypsin on the cast cuticle of *Haemonchus contortus*. Separation of the cuticle was obtained as previously described (*4*). Pepsin was made up in the manner described by Hollaender *et al.* (*5*), and trypsin was made according to the method of Moscona (*6*). The potency of these solutions was tested by using controls containing muscle fibers. Experiments were carried out at 38°C over a period of 3 days, and in no case did hydrolysis of the cuticles occur.

It has been shown that proteins that have a very low aromatic amino acid content, such as gelatin, have a correspondingly slow peptic hydrolysis, and that proteins that contain many aromatic amino acids are hydrolyzed rapidly by pepsin. Recent investigations by Baker (*7*) have shown that dipeptides containing two residues of the *l* form of the aromatic amino acids phenylalanine, tyrosine, and diiodotyrosine are hydrolyzed more rapidly than any previously known synthetic peptide, indicating that the specificity of pepsin is related to the hydrolysis of peptide linkages involving aromatic amino acids. The studies of Hofmann and Bergmann (*8*) on the specificity of trypsin indicate that this is directed to the hydrolysis of peptide bonds involving arginine and lysine.

It has recently been shown that the cuticle of infective larvae of *H. contortus* is composed of proteins, which, however, lack the aromatic amino acids lysine and arginine (*4*). The failure of the proteases to attack the cuticle of the larvae can, therefore, be attributed to the chemical composition of the constituent proteins. It is of further interest to note that more rapid exsheathing rates were obtained in distilled water in which the cuticle had been shown to be soluble at higher temperatures than in solutions of pepsin similar to those used by Crofton (*9*).

ALAN F. BIRD*

Department of Zoology,
University of Adelaide, South Australia

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9. I am indebted to the McMaster Laboratories, Glebe, N.S.W. for supplies of larvae of *H. contortus*, to I. G. Jarrett, C.S.I.R.O., Division of Biochemistry and General Nutrition, Adelaide, for preparing the abomasal fistula, and to the Agricultural Research Council.
* Present address : Department of Zoology, Ashworth Laboratory, Edinburgh 9, Scotland.

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Earthworm Breeding Farms

I have read with interest the news item on earthworm breeding farms [*Science* **120**, 825 (1954)]. It seems necessary that a note of caution be introduced in evaluating the ability of worms to enhance the chemical and physical properties of soil. For example, Chadwick and Bradley [*Proc. Am. Soc. Hort. Sci.* **51**, 552 (1948)] concluded after an extensive series of experiments that although castings are beneficial when added directly to potted plants, the presence of large numbers of worms in the soil had no beneficial results upon plant production. Different species of worms have a strong selectivity for various types of soil.

With regard to this fact it is interesting to note that *Eisenia foetida* is one species that is commonly supplied by worm farms. While easy to culture, the worm is restricted to a habitat of manure or compost, and will die when placed in the loamy soil of the field or garden. Hence any increase in yield from soils to which this species has been added is of a transient nature, being the result of the worms' decomposition