

parts of the mine. The largest pod occurred as a mass 18"-24" thick by 4' wide and 10' long. The massive uraninite, 3"-5" thick, rested on 6"-8" of black vanadium ore commonly designated as corvusite. This was followed by 8 or more inches of a yellow carnotite-type ore, which was topped by 6" of powdery red hewettite.

The specimens of uraninite-bearing ore that have been collected show a black central core of uraninite, in part laced with delicate seams of yellow, orange, and green uranium minerals, and the whole surrounded by carnotite. Although some of these minerals have been tentatively identified, there are many others that have not.

The Bureau of Mines at Salt Lake City, Utah, made a preliminary microscopic and chemical examination of one specimen in August 1951. It consisted of half black oxide and half yellow alteration products. The chemical analysis of the material as a whole gave 66%  $U_3O_8$ , 4.8%  $SiO_2$ , 7.7%  $H_2O$ , and 0.95% copper. There was considerable vanadium present, as well as a minor amount of arsenic. Regarding the identity of the minerals, Harold L. Gibbs states:

The minerals tentatively identified in this small rich sample are uraninite, beta-uranotil, carnotite, and gummite. An X-ray photograph of the black uranium oxide by Allen King of the University of Utah gave the lines of uraninite. The beta-uranotil was observed as thin 100 plates with parallel extinction,  $\gamma = 1.685$ , and strong pleochroism, yellow to colorless. Grains in cold concentrated HCl leave a silica residue. Intergrown with the beta-uranotil in all cases was an extremely fine yellow mineral, index more than 1.74, tentatively identified as carnotite because of the presence of vanadium in the same grain. Two yellow isotropic minerals, one of low index near 1.60 and one near 1.73 are tentatively identified as gummite. The nature of the copper and arsenic occurrence was not identified.

Some of these minerals are new to the mineralogy of the carnotite deposits, and it is expected that others will be found and identified by the U. S. Geological Survey following examination of other specimens from this deposit. When the USGS studies have been completed it is believed that there also will be more information on the nature of the uraninite and its alteration products.

The Grey Dawn carnotite deposit is similar in many respects to other carnotite deposits of the Salt Wash sandstone except for the presence of uraninite, gummite, and  $\beta$ -uranotil. It has individual ore pods that are lenticular, irregular in shape, and essentially parallel to the gentle dip of the sandstone bed. Tiny northwest-trending fractures cross the favorable bed in the vicinity of the ore bodies and are believed to be one of the structures influencing the deposition of uraninite. Although there is no surface indication of the Grey Dawn deposit at the rim outerop, nevertheless some 120' back of the rim a combination of favorable host rock and structure resulted in forming an ore body.

In the carnotite deposits of the Salt Wash sandstone, no original mineral has ever been found the

alteration of which could produce carnotite. The discovery of massive uraninite in the Grey Dawn Mine suggests a source for the uranium, and an unidentified black mineral high in vanadium collected here, as well as in other mines, suggests a source for the vanadium. Not only does the uraninite offer a solution to the origin of carnotite, but it supports the theory that the carnotite-bearing deposits are the product of the alteration of ores of hydrothermal origin. Evidence connecting the deposits with igneous activity is not conclusive, but the presence of the laccolithic La Sal Mountains near the Grey Dawn Mine suggests a close association. The world occurrences of uraninite are listed as associated with heated solutions, and as the Grey Dawn uraninite is not of placer or detrital origin, the possibilities of a hydrothermal origin for the uraninite seem exciting and well worth testing. From the data that are being assembled it appears that a new classification of uraninite-bearing ores is being developed on the Colorado Plateau.

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## An Effect of Proteins and Proteoses on the Cellulase of *Myrothecium verrucaria*<sup>1</sup>

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During work on purification of the cellulase of *Myrothecium verrucaria* Alb. et Schw., strain USDA 1334.2, a marked stimulation of cellulase activity by the addition of proteins and proteoses has been observed. Protein effects on enzymes are well known but the present evidence suggests that the mechanism of the effect on cellulase is unusual.

The enzyme preparation ("Fraction 80") on which the effect has been most studied was obtained from a culture filtrate by concentration and repeated fractional precipitation with ammonium sulfate (1) and stored as a freeze-dried powder. From subsequent evidence its cellulase content has been estimated to be approximately 40% of the total protein. Enzyme activity was assayed from the micro-Somogyi titer of the reducing sugar formed during incubation of enzyme in 20 ml of assay medium for 17 hr at 35° C, with continuous rotary shaking. The assay medium consisted of a 1% dispersion of cellulose substrate in 0.05 M acetate buffer of pH 5.6, with sodium pentachlorophenate as antiseptic. The cellulose substrates have been previously described (1): "precipitated cellulose," a finely

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dispersed, partly degraded cellulose precipitated from sulfuric acid; and "swollen linters," a Wiley-milled cotton linters swollen in alkali under conditions claimed to cause no degradation.

TABLE 1  
EFFECT OF PRELIMINARY INCUBATION  
WITHOUT SUBSTRATE\*

Com- ponents	Albumin (A) added ( $\mu$ g/20 ml)	Titer: ml 0.005 N thiosulfate		
		Precipitated cellulose	Swollen linters	
		pH 5.6	pH 4.8	pH 5.6
$E_{24}$	0	2.58	1.69	2.11
$E_{24}A_0$	80	4.15	7.04	6.12
	320	5.98	8.29	7.70
$E_{24}A_{24}$	80	4.32	8.17	6.24
	320	6.78	9.19	8.40
$E_0$	0	4.37	4.28	4.41
$E_0A_0$	80	6.36	9.52	7.83
	320	7.84	10.12	9.28

\* The subscripts denote hours of incubation before substrate addition. Enzyme (E): 78  $\mu$ g of Fraction 80 protein/20 ml. Titrers for precipitated cellulose are for 1 ml of assay medium; for swollen linters, for 3 ml of assay medium.

Evidence of protein stimulation of cellulase activity was obtained while studying the effects of certain adjuvants on the breakdown of precipitated cellulose by a low enzyme concentration (60  $\mu$ g Fraction 80 protein/20 ml). Cysteine (0.002 M) and constituents of the salts in the *Myrothecium* culture medium (dilution 1:40) had no effect on enzyme reducing sugar titer, but Difco Dubos medium serum (dilution 1:1500) approximately doubled it. Controls of substrate and serum alone and of enzyme and serum alone had negligible reducing sugar titers. No effect was given by autoclaved serum, by the fraction of serum not precipitable by 2.5% trichloroacetic acid, or by sulfated serum ash, suggesting that serum protein was responsible for enzyme stimulation. Addition of 1 mg of the following proteins gave a similar stimulation: crystalline bovine plasma albumin (Armour), crystalline  $\beta$ -lactoglobulin (Armour), crystalline pepsin (Armour), crystalline lysozyme (Armour), and gelatin (Difco). The nondialyzable fraction of Difco proteose-peptone, a product free of protein on the evidence of precipitation tests, was equally effective. Difco peptone and Difco tryptone had no effect. Thymonucleic acid was also inactive.

Under the above conditions, added protein stimulated enzyme activity toward insoluble substrates only. These included precipitated cellulose, swollen linters, and unswollen linters. Protein addition had no effect on enzyme activity toward cellobiose or the soluble carboxymethyl cellulose (Type 50T, Hercules Powder Co.) whose enzymatic breakdown has been studied by Reese *et al.* (2, 3). The effect with insoluble substrates was not peculiar to Fraction 80 but was also shown with an untreated culture filtrate as enzyme source. Stimulation was not significantly affected by dialyzing the enzyme against purified buffers, by purifying or

washing the components of the assay medium, or, under sterile conditions, by removing the antiseptic of the assay medium. Trials were made of the effect of added protein on the enzyme during a preliminary incubation, under the same conditions as for assay, in the absence of substrate. The results for 24-hr incubation before substrate addition (Table 1) show that, although the titer of enzyme without added protein was markedly reduced by this treatment ( $E_{24}$ ,  $E_0$  series), the increase in titer from protein added at the start of the preliminary incubation period was not greatly different from that of protein added at the end ( $E_{24}A_{24}$ ,  $E_{24}A_0$  series). With less prolonged periods (17 hr) the differences were still less. It was concluded that the effect of added protein was not primarily due to a protection of the enzyme against denaturation or against an inhibitor present in the assay medium or the enzyme preparation.

For a given level of added protein, the extent of cellulase stimulation depended on at least four factors. (1) It varied with pH (Fig. 1), the variation for higher protein levels being sufficient to change the apparent pH optimum of the enzyme for both precipitated cellulose and swollen linters. This change did not appear to depend on the isoelectric point of the protein added, for, with both substrates, the curves for lysozyme (100  $\mu$ g) from pH 4.0 to 7.2 and for pepsin (1 mg) from pH 5.6 to 7.2 were closely similar to curve 3 for albumin. (2) Stimulation varied with temperature, the percentage stimulation by albumin at 27° C being more than double that at 35° C with both substrates. (3) Percentage stimulation increased with increasing substrate concentration (Fig. 2), the increase continuing beyond a substrate level (1%) which, in the absence of added protein, was rate-limiting only for high enzyme concentrations. (4) Stimulation decreased with increasing enzyme concentration, an effect which was possibly due to a sparing action by noncellulase protein in the enzyme prepara-

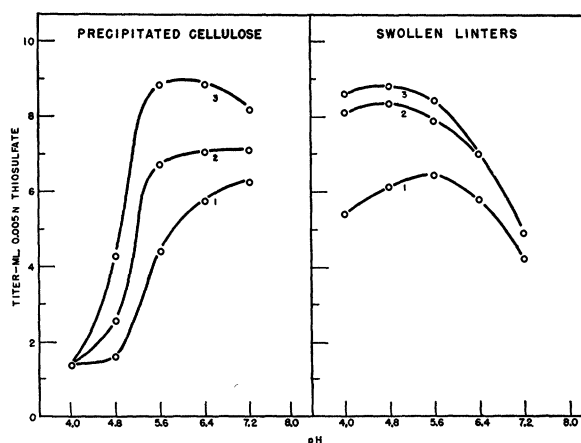


FIG. 1. Effect of pH on cellulase stimulation by albumin. Enzyme: 78  $\mu$ g Fraction 80 protein. Albumin: curve 1, 0; curve 2, 60  $\mu$ g; curve 3, 240  $\mu$ g. Buffers: pH 4.0–5.6, 0.05 M acetate; pH 6.4–7.2, 0.025 M phosphate. Titrers: precipitated cellulose, per 1 ml of assay medium; swollen linters, per 3 ml assay medium.

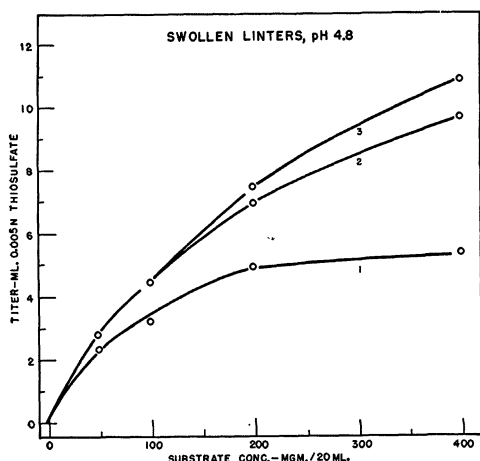


FIG. 2. Effect of substrate concentration on cellulase stimulation by albumin. Enzyme: 66  $\mu$ g Fraction 80 protein. Albumin: curve 1, 0; curve 2, 56  $\mu$ g; curve 3, 220 and 440  $\mu$ g. Buffer: 0.05 *M* acetate. Titer: per 3 ml assay medium.

tion. These effects may explain the present confusion in the literature (4) on such properties of crude cellulase preparations as pH optima for different substrates.

Evidence that added protein was partially adsorbed on the substrate was obtained as follows. Before enzyme addition, albumin and substrate (400 mg) were incubated in 19 ml of assay medium for 1 hr and then centrifuged until the volume of the sediment was less than 10% of the total volume. The supernatant solution was replaced by fresh buffer, and the enzyme titer in the resulting medium compared with that in uncentrifuged controls. The titers for precipitated cellulose at pH 5.6 (Table 2) corresponded to about 50% retention of protein by the centrifuged substrate; for swollen linters they were less, corresponding to about 25% retention.

The present data are considered to suggest that cellulase stimulation by protein is due to protein adsorbed on the substrate. A mechanism dependent on adsorption would, as required, be confined to insoluble substrates and be sensitive to low concentrations of added protein and to differences in substrate proper-

TABLE 2  
EFFECT ON PROTEIN STIMULATION OF REMOVAL OF  
ALBUMIN UNADSORBED ON PRECIPITATED  
CELLULOSE\*

Albumin added ( $\mu$ g/20 ml)	Titer: ml 0.005 <i>N</i> thiosulfate/ml assay medium	
	Control series (uncentrifuged)	Centrifuged series
450	9.07	8.65
220	8.75	8.17
110	8.03	7.45
55	7.61	6.18
28	6.28	5.86
0	4.63	—

\* Enzyme: 68  $\mu$ g Fraction 80 protein.

ties. It also permits a simple interpretation of the effect of increasing the substrate concentration, for this would increase the extent of adsorption. However, no conclusive evidence has yet been obtained to indicate the manner in which adsorbed protein exerts its effect.

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## Selective Stimulation of Color Receptors with Alternating Currents

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A sensation of flickering phosphenes is aroused by an alternating current passing through the head. With this sensation as an index, much work has been done to determine threshold strengths of currents as a function of frequencies (1-5). Two examples of strength-frequency curves obtained in our laboratory are illustrated in Fig. 1. In these experiments the stimulating

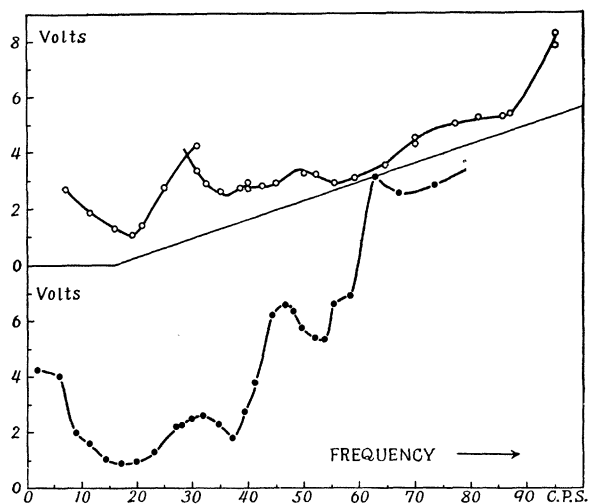


FIG. 1. Strength-frequency curves of moderately light-adapted human eye. Sinusoidal alternating currents and rectangular pulses of varying frequencies were used to obtain upper and lower curves, respectively.

voltage was raised at an approximately constant rate of about 80 mv/sec until the subject perceived the appearance of flicker, and then lowered from a high level sufficient to evoke a sensation of distinct flicker until the subject noticed the disappearance of flicker. The average of both values was taken as the threshold strength.

In another series of experiments a constant voltage