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STRATOSPHERIC CLOUD

4. J. E. Stein and J. G. Denison, *Proc. Natl. Shellfisheries Assoc.* **50**, 67 (1961).
5. J. R. Uzmann and J. W. Jesse, *Progressive Fish Culturist*, in press.
6. D. V. Zander, thesis, Univ. of California, Davis (1953).
7. E. Moore, *Trans. Am. Fisheries Soc.* **52**, 74 (1922); D. H. Weinrich, *J. Parasitol.* **33**, 62 (1947); W. F. Hughes and D. V. Zander, *Poultry Sci.* **33**, 810 (1954).
8. A. Bishop, *Parasitology* **25**, 163 (1933).
9. Difco Laboratories Inc., Detroit, Mich.
10. Microbiological Associates, Inc., Washington, D.C.
11. Nutritional Biochemicals Corp., Cleveland, Ohio.
12. Sigma Chemical Co., St. Louis, Mo.

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"Applejack" Technique: New Application of an Old Approach to Solute Concentration

Abstract. *Freezing and slow thawing is a simple and inexpensive means for preparing concentrated solutions from large volumes of water-soluble compounds. Particularly appropriate for colored compounds, it can be adapted easily to colorless solutes for which quantitative tests are available.*

The purification of compounds from plant or animal sources often uses techniques such as dialysis, chromatography, and electrophoresis. These procedures dilute the products in the course of their separation from contaminants in the crude extracts.

The extremely simple technique described here has been used to concentrate the blue-green plant pigment, allagochrome (*1*). Solutions which have been completely frozen in plastic bottles in a deep freeze or dry-ice chest are transferred to a cold room (3° to 4°C) and supported in an inverted position above a collecting container. A several-fold concentration of the solute is obtained in the first effluent. When containers are placed on blocks of dry-ice, freezing is more rapid than in a deep freeze and samples freeze from the bottom up. The solute is thus most concentrated in the last portions of the solution to freeze where it is in a position to drain off first on melting. Maintaining a low constant temperature during thawing permits sufficient equilibration between the outside and the inside of the container so that melting (accompanied by extensive channeling) occurs throughout the sample rather than just in those parts adjacent to the bottle wall.

Table 1 summarizes measurements of the optical density of consecutive 20-ml fractions from three different 500-ml samples of allagochrome of known initial concentrations. Data on the concentration of hemoglobin and flavin mononucleotide are also included. Although the latter compounds exhibited rather unusual patterns of concentration in the effluent fractions, at least a twofold increase in concentration is possible by visually selecting that portion of the melted sample of obviously darker color. Other means would have to be used to estimate the concentrations of colorless solutions.

Between six- and seven-fold concentrations of allagochrome and quantitative recovery of solute have been achieved by this method. Routinely, the melting sample is collected in two fractions: the first 25 percent which contains the major portion of the solute, and the 25 to 80 percent fraction which contains the rest. The second fraction is recycled with a new sample. The ice remaining in the freezing bottle is discarded.

This technique is available to anyone with access to a deep freeze or dry-ice chest and a refrigerator. It provides an economical method for concentrating large volumes of water-soluble materials in laboratories where the facilities for lyophilization are limited.

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Table 1. Relative concentration (optical density) of consecutive 20-ml fractions collected from 500-ml frozen samples. Fractions were collected on an automatic fraction collector with a 20-ml volumetric siphon. Spectra were measured with a Beckman DK-2 recording spectrophotometer. Initial concentrations were as follows: allagochrome, 0.89, 2.18, and 3.01 mg of dried sample per milliliter; flavin mononucleotide (FMN), 0.01 mg/ml; hemoglobin, 0.17 mg/ml.

Allagochrome (673 mμ) at initial concentration			Hemoglobin (406 mμ)	FMN (444 mμ)
0.89	2.18	3.01		
		Initial sample		
0.58	1.42	1.96	1.20	0.24
		Fractions 1 to 7		
2.18	9.50	9.45	0.57	0.39
2.08		6.06	1.20	0.22
	4.56*			
1.99		5.25	1.46	0.26
1.62	2.60	4.85		0.39
			2.62*	
1.35	2.47			0.54
		3.97*		
1.03	2.08		2.66	0.66
0.86	1.77	3.00	0.93	0.61
		Average		
1.59	3.93	5.33	1.72	0.44

* Average for two consecutive fractions.

References and Notes

1. H. M. Habermann, in *Comparative Biochemistry of Photoreactive Systems*, M. B. Allen, Ed. (Academic Press, New York, 1960), pp. 73–82; ———, in *Progress in Photobiology*, B. C. Christensen and B. Buchman, Eds. (Elsevier, Amsterdam, 1961), pp. 576–580.
2. Supported by grants from the U.S. Public Health Service (GM 07659-03) and the National Science Foundation (G 17656).

15 February 1963

Stratospheric Cloud over Northern Arizona

Abstract. *An unusual ring-shaped cloud was widely observed over northern Arizona near sunset on 28 February 1963. From a large number of observers' reports it is known to have appeared overhead near Flagstaff, Arizona. From initial computations based on four photos taken in Tucson, 190 miles south of the cloud, its altitude was approximately 35 kilometers. The most distant observation reported was made 280 miles from the cloud. The cloud remained sunlit for 28 minutes after local sunset. Iridescence was noted by many observers. Tentatively, the cloud may be regarded as similar to a nacreous cloud; but its unusually great height and unusually low altitude, plus its remarkable shape, suggest that it was a cloud of previously unrecorded type.*

Near sunset, on 28 February 1963, a cloud of unusual configuration and coloration was observed in widely scattered localities in Arizona and some surrounding states. The cloud took the form of a large oval ring (clear in the middle) with the long axis running north and south (Fig. 1 and cover photograph, this issue). It remained brightly illuminated well after the sun had set on high cirrus clouds to the west. From Tucson, 190 miles to the south, its angular elevation appeared to be about 6 degrees. A rough computation of its height, based on sunset geometry (*1*), made immediately after the cloud entered the earth's shadow, led me to appeal by press and radio for confirmatory reports in order to establish the approximate location and to secure descriptions from the largest possible number of other observers.

From approximately 150 reports, many communicated by persons well aware that they had seen a type of cloud unprecedented in years of sky-watching, it was quickly established that the cloud lay overhead in the vicinity of Flagstaff, Arizona, that it exhibited iridescence of the sort asso-

ciated with stratospheric nacreous clouds in the arctic (*2*, *3*), and that its internal structure was very peculiar. To observers nearly underneath, the colors green and blue were visible, and a pinkish cast was noted at times. A fibrous texture, described by several independent observers as resembling a "wood grain" appearance, was present over much of its northern extent, but its southern end was denser and more cumuliform. Its overall shape was compared by some (ranchers) to a horseshoe or a horsecollar if it was viewed from the south; from the north it appeared as a closed loop with a long thin trail that could be seen extending northward, from the oval, and several observers in that sector compared its shape with that of a "hangman's noose." The cloud was seen from distances as great as 280 miles (near Douglas, Arizona, and Albuquerque, New Mexico, respectively).

Many observers reported a second cloud off to the northwest of the main cloud, with shape very much like that of the main cloud, but only about a quarter as large. Correctness of these reports has been established from some of the first photographs that have come in from northern Arizona. The cloud was evidently moving generally southeastward, though visual reports are in some conflict on this point; this point can only be resolved from further studies by triangulation.

By fortunate coincidence, the cloud appeared within a few tens of miles of the U.S. Weather Bureau radiosonde station at Winslow, Arizona, and a high-altitude sounding had been completed there only an hour before the appearance of the cloud. A jet stream lay almost directly under the cloud and over Flagstaff, and there were peak winds of 98 knots from the northwest occurring over Winslow at an altitude of about 11 kilometers. The radiosonde run terminated at the 13-millibar level of atmospheric pressure (about 29 km), where the temperature was −46°C. There was very little direction shear in the Winslow wind sounding, a condition known to favor formation of mountain waves and believed to be conducive to nacreous clouds, at least in Scandinavia (*2*). It is possible, therefore, that the San Francisco Peaks just north of Flagstaff disturbed the flow so that wave motion was set up in the stratosphere, but this remains a conjecture, pending further study of reports of first appearance. Whereas

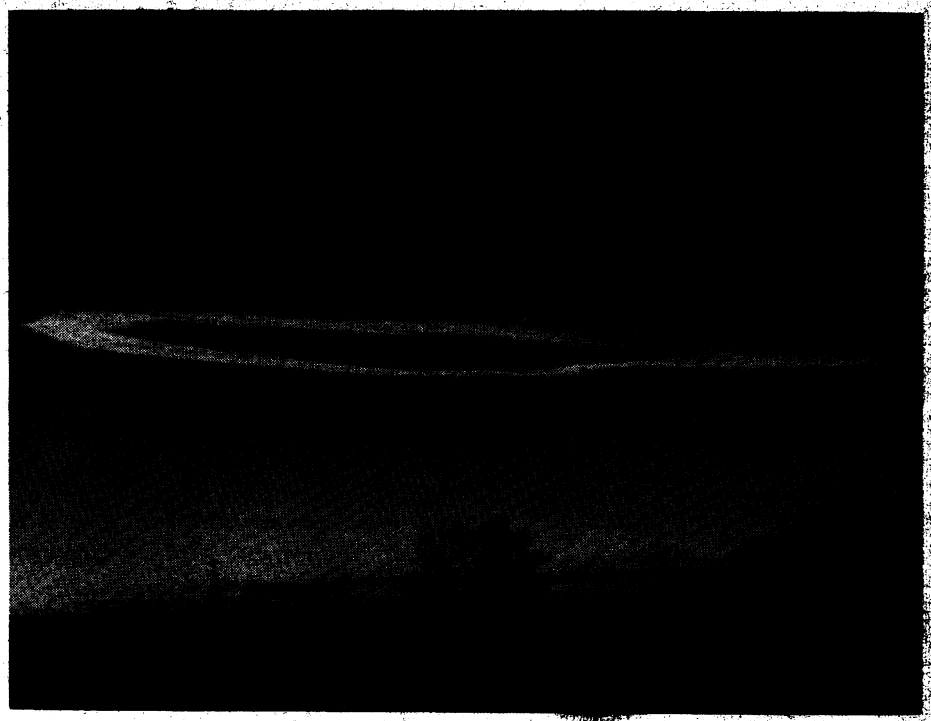

Fig. 1. Stratospheric cloud over Flagstaff, Arizona, from a point about 160 miles east-southeast, after sunset. The dark clouds in the west are cirrus clouds on which the sun has already set. [I. E. Daniels, Springerville, Arizona]

some recent studies (*4*) suggest strong local stratospheric cooling as a prerequisite for the formation of nacreous clouds, the sounding at Winslow showed little departure from average temperature conditions in the lower and middle stratosphere.

Photogrammetric analysis of the four photographs known to have been taken in the Tucson area have yielded elevation angles of the near point ranging from 5.9 to 6.2 degrees. Because the exact range to the nearest point of the cloud is not yet known to better than about 10 or 15 miles in 190 miles, the exact height cannot yet be determined. However, the cited elevation angles plus allowance for earth curvature give a cloud height of 35 kilometers, possibly a bit higher if the range to the near point proves to be greater than 190 miles. This height is distinctly greater than that of reported Scandinavian nacreous clouds. Photogrammetric heights obtained over many years by Størmer and others (*2*, *3*) are no higher than 30 kilometers, and the majority lie between 22 and 28 kilometers.

The estimated height of 35 kilometers rules out the possibility that the Flagstaff cloud could have been the condensation trail from a jet plane. The present American altitude record, made under the most favorable conditions directly above the home field by a Lockheed F-104 in 1959, is 103,395 feet (31.6 kilometers). Perhaps more conclusive is the fact that the upper limit of height for possible contrail formation (*5*) as indicated by the sounding from Winslow was just under 24 kilometers at the time of the cloud's appearance.

These preliminary indications mark the Flagstaff cloud of 28 February as a most unusual phenomenon of considerable meteorological interest. Requests for photographs, still being made at time of this writing, have already brought promises of photographs from a total of 16 sites reasonably well dispersed around Arizona, so fairly precise data on the cloud's height, shape, and dimensions should be obtainable by triangulation. A conflict between heights estimated from the Tucson photos and from sunset geometry is under study (the indicated height based on available reports of fadeout time is about 25 kilometers). Premature fadeout may have been due to cirrus clouds between the cloud and the ray-tangency point, computed to lie at or very near Los Angeles.

The hydrodynamics of the field of vertical motion that produced such a toroidal cloud form are very puzzling. Present estimates give the closed oval a length of about 60 kilometers and a

width of about 30 kilometers, with a ring cross section of perhaps 3 to 4 kilometers in the horizontal. I am not aware that a cloud of such form and size has been observed at any level within the atmosphere before. Interesting questions about the source of the requisite water vapor are posed by its unprecedented altitude (*6*).

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References and Notes

1. S. K. Mitra, *The Upper Atmosphere* (Asiatic Society, Calcutta, ed. 2, 1952).
2. E. Hesstvedt, *Geofys. Publikasjoner Norske Videnskaps. Akad. Oslo* **20**, No. 10 (1959).
3. A. Y. Driving, *Bull. Acad. Sci. U.S.S.R. Geophys. Ser. 3, English Transl.* (1959), pp. 279–286.
4. Y. Gotaas, *Geofys. Publikasjoner Norske Videnskaps Akad. Oslo* **22**, No. 4 (1961); A. Y. Driving and A. I. Smirnova, *Bull. Acad. Sci. U.S.S.R. Geophys. Ser. 3, Eng. Transl.* (1958), pp. 187–191.
5. H. Appleman, *Bull. Am. Meteorol. Soc.* **34**, 14 (1953).
6. I thank Leon Salanave for alerting me to the cloud when it became visible in the Tucson sky and for further technical assistance, and I. E. Daniels and C. E. Peterson for permission to reproduce their photographs. The cooperation of the numerous Arizonans submitting reports is gratefully acknowledged. Supported by the Office of Naval Research under contract NR 082–164.

20 March 1963

Potentiation by Adrenaline of a Proteolytic Activity Associated with Purified Myosin

Abstract. *Proteolytic activity accompanies myosin through three reprecipitations. The fact that this activity can be potentiated by very small doses of* l*-adrenaline supports the view that adrenaline receptors are protein in nature and that adrenaline-like compounds exert their action through modification of the activity of enzymes.*

Correlation of certain disparate experimental observations led us to examine muscle myofibrillar elements for a catheptic protease activated by adrenaline. Elevated levels of adrenaline and increased catheptic activity have been observed in dystrophic mouse muscle (*1*, *2*). Catheptic activity has been noted in isolated myofibrils (*3*), in crude myosin (*4*), in actin preparations (*5*), and in a KCl extractable fraction of muscle (*6*). In physiological experiments, adrenaline has increased the amino acid levels in plasma of eviscerated rats (*7*) and under other circumstances has increased the output of urinary nonprotein nitrogen (*8*).

Preliminary experimentation revealed proteolytic activity in crude preparations of rabbit myosin that could be stimulated by adrenaline. We purified the myosin in these preparations by repeated reprecipitation, according to procedures described by Mommaerts (*9*), and studied the effect of *l*-adrenaline and *d*-adrenaline on this activity after the third cycle. Thus, crude myosin was separated from whole muscle homogenate by extraction with cold Weber's solution and was subjected to three subsequent cyclings involving selective solubilization and precipitation via time- and temperature-controlled manipulation of ionic strength (*9*). Each myosin fraction manifested proteolytic activity at *p*H 3.5, 5.0, and 6.0; however, we are reporting only on the work carried out at *p*H 3.5, thus delimiting the protease examined as a cathepsin. The enzyme assay employed 0.4*M* acetate buffer, *p*H 3.5, and hemoglobin as substrate. The concentration of adrenaline varied from 10 to 10^{-6} μg/ml. Increase in free tyrosine during incubation at 35°C served to define proteolytic activity. Incubation times varied from 5 minutes to 2 hours. Tyrosine was measured by fluorimetry after it had been conjugated with nitrosonaphthol and had formed the yellow fluorophore (*10*). This method permits separation of tyrosine from other compounds including adrenaline which fluoresce in the ultraviolet and allows data to be obtained on the relationship between concentration and fluorescence intensity at tyrosine concentrations too dilute for assay by traditional ultraviolet or visible absorption methods.

Release of tyrosine linear with time occurred with the crude myosin fraction. In five of seven experiments with 2 to 10 μg of adrenaline per milliliter enhancement was observed as a change in slope of this line, the increase lying between 40 and 200 percent. On the other hand, 5 μg/ml of adrenochrome did not cause potentiation. In two of seven experiments the killing of the rabbit was not rapid. In extracts prepared from these animals the endogenous cathepsin was initially high and adrenaline did not increase the activity. This phenomenon was occasionally observed in the purified fractions as well.

In Figs. 1 and 2 we have charted the results of some of our studies. Tubes were incubated for 60 minutes and analyzed in duplicate; trichloroacetic acid was employed to stop the reaction. All incubations were run at

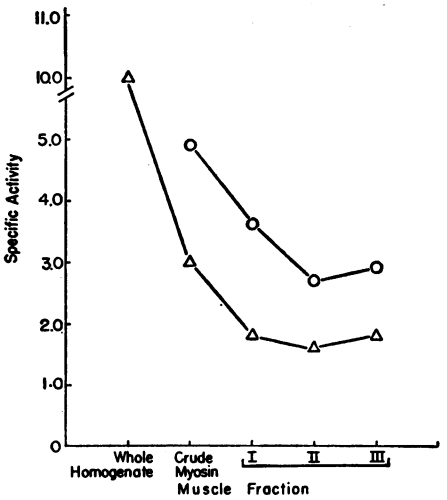

Fig. 1. Change in specific catheptic activity (micrograms of tyrosine released per milligram of protein in a 60-minute incubation) with separation and progressive purification of the muscle myosin fraction. Incubations were performed at two concentrations of enzyme protein. Triangles: 1.1 mg of protein per milliliter; circles: 0.59 mg of protein per milliliter.

one of two constant enzyme concentrations, 1:5 dilutions of two stocks containing 1.1 and 0.59 mg of protein per milliliter.

The work of Nardone (*4*) suggested the association of a proteolytic activity with myosin. Our preparations had an activity and solubility minimum in preliminary studies at about *p*H 5.0 which is close to the isoelectric point of myosin. Our methods, though they produce physical crystallinity, fall short of producing more than 90 percent homogeneity of the proteins by electrophoretic analysis (*9*). Thus, whether the enzyme activity described represents a function intrinsic to myosin or that of a contaminant molecule cannot be settled now.

What may be the meaning of the initial loss in specific activity we ob-

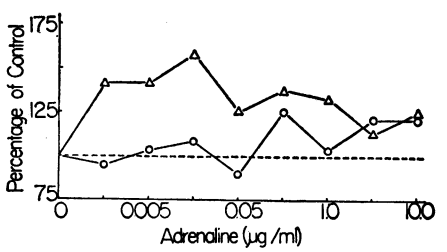

Fig. 2. Effect of *l*-adrenaline (triangles) and *d*-adrenaline (circles) on the catheptic activity of myosin fraction reprecipitated three times, expressed as percentage of control.

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