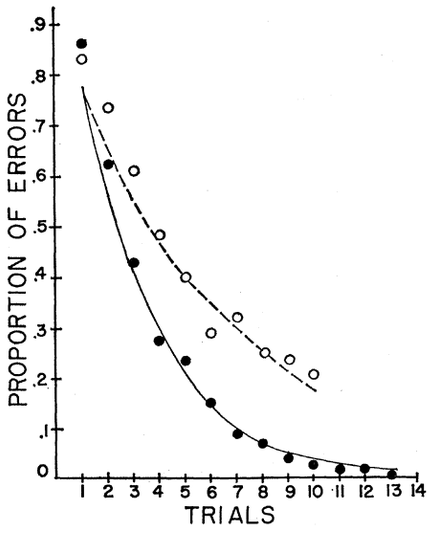

Fig. 1. Proportion of errors made by experimental subjects (open circles) and controls (solid circles). The smooth curves are the predictions derived from the two-stage model.

practice trials with a six-syllable list until they reached a criterion of one errorless trial.

The most important results of this experiment are presented in Fig. 1. The substitution procedure retarded learning in the amount predicted by the two-stage model. The theoretical curves in Fig. 1 were obtained by estimating the parameters of the model from the data of the control group ($c = .35$, $s = .55$, $e = .17$, $p = .61$). The predicted mean learning curve for the experimental group was then computed by inserting these values into Q^{*n}. Considering that no data from the experimental group were used for this prediction, the fit is quite good. The occurrence of more errors than predicted during the first few trials in both the control and experimental groups might possibly be due to a warm-up effect in the experiment because of insufficient pretraining.

The probability of an item being replaced after one or more correct responses was .29; predicted was .24.

Apart from predictions concerning the experimental group, the model can also be used to describe the behavior of the control group. The crucial point here is the prediction that successes in state C_1 will be independent events, emitted with a stationary probability p. On trials between the first success and the last error subjects must be in state C_1. A Vincentized learning curve based upon responses on these trials reveals that the proportion of errors decreases from .39 in the first half of the trials to .36 in the second, which is far from being statistically significant ($\chi^2 = .12$). A test for independence also led to a nonsignificant χ^2 value ($\chi^2 = 1.47$, one degree of freedom). These results are in good agreement with the model but they are based upon too small a portion of the data, since, owing to the generally fast learning, many items did not have any responses between the first success and last error. Statistics which are based upon all items with their predictions derived from the two-stage model are given in Table 1.

The results of the present experiment were completely confirmed in a second experiment. The two-stage model predicted 5.09 errors per item during the first 10 trials in the substitution condition; 5.16 were observed. Again, this is a true prediction, since no data of the experimental group were used for parameter estimation. The only difference between the two experiments was that in the second experiment subjects were not required to read all items aloud. This experiment was therefore more open to the criticism that any observed all-or-none behavior simply reflected the fact that subjects could concentrate upon a few items at a time.

In spite of the successful predictions derived from the present model, a major problem remains. What has proved its usefulness here is a two-stage Markov model. The interpretation given to the two stages in terms of response learning and associative stages has not been tested directly. In order to do this, it would be necessary to show that the parameters of the model are differentially affected by experimental manipulations of the amount of response learning required and the difficulty of association (*9*).

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7. The expressions used are the mean number of errors, the mean trials before the first success, and the mean number of times a success is followed by an error. For details of the estimation procedure as well as for the derivation of other predictions see Bower and Theios (*6*).
8. This procedure resulted in quite a homogeneous item pool. Only very little evidence of item selection due to differential difficulty was found in the present experiment.
9. The assistance of Jacqueline Bragg and Don McCoy in collecting part of the data is acknowledged. This research was supported by NSF grant GB-195.

11 February 1963

Evaporation Enhancement by Protein Films

Abstract. *Evaporation of water takes place more rapidly from buffered solutions of ovalbumin or hemoglobin than from solutions of certain surface-active agents or from buffer alone. The effect appears to be connected with adsorption of protein.*

It is well known that films of surface-active substances can reduce the evaporation of water. Practical use is made of such films in large-scale projects for slowing water loss from reservoirs by application of monolayers of hexadecanol or similar substances (*1*). In addition, Deryagin *et al.* have shown mathematically and experimentally that surface films can actually increase the evaporation rate of water under certain special conditions (*2*). Furthermore, Bull found that constantly renewed adsorbed films of ovalbumin appeared to increase greatly the evaporation of water (*3*). He found no significant effects when the surfaces were motionless, however. The latter experiment was carried out by placing dishes of water and protein solutions overnight in a desiccator, with evaporation rates being determined from the loss in weight of the dishes and their contents (*4*).

Langmuir and Schaefer have discussed evaporation as a kinetic process involving successive steps (*5*). These are transport of water molecules to the surface, passage across the surface region, and diffusion through a quiescent layer of air overlying the liquid surface. Archer and LaMer have considered the energy barriers to each of these processes in terms of a number of resistances (*6*). One result of this analysis is that if a single resistance is much greater than the others, it com-

pletely overshadows the rest in determining the overall rate of the evaporation process. Thus experiments in desiccators or with adsorbents placed above solution surfaces risk the introduction of a diffusional resistance completely obscuring surface effects. The experiments to be described here were planned with the objective of lowering the diffusional resistance. Evaporation rates were measured by determining the loss in weight from thin films of solution contained on wire rings or porcelain disks suspended in slowly moving air of controlled humidity. This direct approach utilizing slow evaporation from virtually stationary surfaces gave results qualitatively similar to those found by Bull for continuously formed protein films.

For surface-active solutes such as proteins or synthetic detergents at sufficiently high concentrations, evaporation was measured from thin liquid layers formed by dipping platinum wire rings of the type used on the duNouy tensiometer into the solutions and slowly withdrawing them. The loss in weight due to evaporation was measured beginning 20 to 40 seconds after dipping by suspending the rings from the hook of a Mettler type H-15 single-pan balance and taking readings at either 10- or 40-second intervals over a period of 280 seconds. During the entire period of 280 seconds the beam was released and the balance with its doors left open was covered by a rectangular plastic bell cover (20 by 12 by 20 inches). The bell cover was raised about ¾ inch above the bench top to allow a very limited circulation of air within the balance case. The experiments were carried out in rooms in which circulating air was maintained at relative humidities between 40 and 50 percent and at temperatures of 22° to 25°C. In this work evaporation was a linear function of time and the measured rates were corrected to 50 percent relative humidity by assuming an inverse linear proportionality between rate and relative humidity; this appears to be justified, from examination of the literature (*7*).

In order to measure evaporation from solutions of the surface-active materials at very low concentrations or from the buffers alone, it was necessary to adopt another technique. Since films could not be stabilized on the wire ring under these conditions, perforated porcelain Gooch filtering disks were dipped into the solution and, after removal of most of the excess solution on the disks, were suspended from the balance hook by means of wire frames. The loss in weight due to evaporation from the solution adhering to the disks was then measured and corrected to 50 percent relative humidity in the same way as before.

Table 1. Rates of evaporation from ring and disk, and average errors of the rates: $a = \pm(1/n)\Sigma(d)$. Number of trials shown in parentheses.

Solution	Evaporation rates (10^{-3} mg sec^{-1} cm^{-2})	
	From ring	From disk
Ovalbumin (0.1%)	3.24 ± 0.05 (5)	3.08 ± 0.14 (6)
(primary film)	2.98 ± 0.05 (7)	2.77 ± 0.02 (5)
(aged film)	2.88 ± 0.16 (9)	
Ovalbumin (0.5%)	2.96 ± 0.02 (5)	
Ovalbumin (1.0%)	2.95 ± 0.22 (8)	
Hemoglobin (0.1%) in phosphate	3.24 ± 0.10 (5)	
Alipal CO–436 (0.01%)	2.79 ± 0.07 (5)	2.66 ± 0.05 (5)
Alipal CO–436 (0.1%)	2.68 ± 0.10 (4)	2.51 ± 0.06 (6)
Same, in phosphate	2.59 ± 0.12 (6)	
Igepal CO–630 (0.01%)	2.76 ± 0.05 (5)	2.61 ± 0.04 (4)
Sodium dodecyl sulfate (0.005%)		2.64 ± 0.05 (5)
Acetate buffer	2.79 ± 0.13*	2.63 ± 0.12 (9)

* Calculated from experiments employing porcelain disk.

The average rates of evaporation from solutions of several proteins and common surface-active agents are listed in Table 1. Unless otherwise indicated the solutions were pipetted 15 minutes before beginning the experimental run, with particular care taken to avoid transferring any of the surface film formed during dissolution of the proteins. The solutions, with two exceptions, were prepared in acetate buffer, 0.1 ionic strength, *p*H 5.1. As noted in the table, two solutes were dissolved in phosphate buffer, 0.1 ionic strength, *p*H 7.5. Alipal CO-436, an ammonium salt of the sulfate ester of an alkylphenoxypoly(ethyleneoxy)ethanol, and Igepal CO-630, a nonylphenoxypoly (ethyleneoxy)ethanol, were commercial products of the General Aniline and Film Corp. and were weighed out directly from the samples furnished, which had approximately 60 and 100 percent activity, respectively. The ovalbumin and hemoglobin used were 2X crystalline preparations from Worthington Biochemical Corp. and Nutritional Biochemicals Corp.

Evaporation rates at 50 percent relative humidity are tabulated in units of 10^{-3} mg sec^{-1} cm^{-2} of liquid surface area. The areas were determined geometrically and are thus somewhat approximate although quite constant for the measurements involving the ring. The surface areas of films on the disk are much more approximate, having been estimated from the size of the disk and an assumption of concave hemispherical liquid surfaces in each of the small holes of the porcelain disk. The weights of liquid wetting the disk in each experiment were restricted to the range of 0.09 to 0.15 g in an effort to maintain a relatively constant surface area. The areas calculated for the films on the ring and disk were 5.80 cm^2 and 9.92 cm^2, respectively.

An examination of the data in Table 1 shows that there is good qualitative agreement between the relative rates measured by use of the ring or the disk. Where comparison is possible, the values from the ring were larger by a factor of 1.06. Employing this factor, a hypothetical rate of $2.79 \times 10^{-3} \pm 0.13$ mg sec^{-1} cm^{-2} was obtained for a film on the ring composed solely of acetate buffer solution.

The surface-active agents examined showed negligible effects upon evaporation rate. However, evaporation took place significantly faster from 0.1 percent solutions of ovalbumin or hemoglobin than from the buffer alone or from solutions of the surface-active agents. When the ovalbumin concentration was increased to 0.5 percent, or the film adsorbed on the surface of the 0.1 percent ovalbumin allowed to stand for 1 hour (the aged film of Table 1), instead of the usual 15-minute period, before dipping the ring, the rates of evaporation were reduced to values not significantly different from that estimated for the buffer alone. The rates of evaporation from the ring or disk which had been dipped through the surface film formed during the original dissolving of the protein (the primary film) were also slower than the rates found for recently adsorbed films. These primary films contained a visible coagulum of ovalbumin.

The evaporation rates reported here are certainly low, as they were in the much-criticized work of Hedestrand

(*8*). However, where his experimental arrangement included over the surface a layer of static air which provided a sufficient barrier to evaporation to give the erroneous conclusion that surface films were without effect (*1*), the current experiments permitted the liquid surfaces to be freely accessible to air currents and thus decreased the barrier.

No wholly satisfactory explanation of the action of adsorbed layers of protein to increase evaporation from their solutions is available. The fact that workers studying evaporation through spread monolayers of protein have not reported any increased evaporation, even when moving currents of air were employed (*9*), suggests that the situation may be different with monolayers. Enhanced evaporation appeared in Bull's work with continuously generated adsorbed layers or in the present work with newly formed absorbed films. In both cases the surface film was not in equilibrium with the bulk solution and protein was still adsorbing during evaporation. Where adsorption was virtually complete owing to long surface aging, or at higher protein concentrations where adsorption was completed more quickly, there was less enhancement of evaporation. This seems to imply that continuing adsorption of protein is related to the increase in evaporation rate, as was first suggested by Bull (*3*).

Globular protein molecules have a considerable amount of water associated with them, and the process of surface denaturation, in common with other types of denaturation, produces a decreased binding of water. Hence, adsorption of protein followed by denaturation at the surface could serve to transport a small excess of water to the surface region. However, as was pointed out (*3*), this amount of water is insufficient to account for the increased evaporation found.

In addition, Tovbin and Savinova (*10*) have investigated the nonsteady-state kinetics of water evaporation and have shown that at very short times of contact between water and a jet of moving air evaporation was enhanced. With increasing time of phase contact evaporation rates at first increased and then decreased, approaching steady-state values. In the present work the times of contact between moving air and the liquid surface, while impossible to assess, are nevertheless probably rather short (*11*).

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18 February 1963

Reticulocyte Protein Synthesis: Response of Ribosome Fractions to Polyuridylic Acid

Abstract. *Reticulocyte ribosomes with sedimentation coefficients greater than 100S ("heavy" ribosomes) appear to be considerably more active in hemoglobin synthesis than are 78S ribosomes. When assayed for the ability to synthesize polyphenylalanine in the presence of polyuridylic acid, the 78S ribosomes and "heavy" ribosomes have similar activities. Polyuridylic acid inhibits incorporation by "heavy" ribosomes of amino acids other than phenylalanine.*

Protein synthesis in rabbit reticulocytes occurs on "heavy" ribosomes that have a sedimentation coefficient greater than 100*S* (*1*). Ribosomes with a sedimentation coefficient of 78*S* comprise the major portion of the isolated ribosomes and do not appear to function in protein formation. Electron micrographs indicate that "heavy" ribosomes may be clusters of two or more 78*S* ribosomes (*2*). Polyuridylic acid (polyU), as well as other synthetic polyribonucleotides, can direct polypeptide synthesis on microsomes and ribosomes of a variety of mammalian cells (*3–5*). That this is true of ribosomes obtained from reticulocytes (*3, 5*) is of particular interest, since the formation of hemoglobin, which accounts for over 80 percent of the protein synthesized in these cells, is directed by a relatively stable form of RNA (*1, 6*). This study was undertaken to determine which class of reticulocyte ribosomes participates in the incorporation of phenylalanine into polyphenylalanine when this synthesis is directed by polyuridylic acid.

Reticulocytes were prepared from phenylhydrazine-treated rabbits (*7*). The cells were lysed either by two cycles of freezing and thawing (*3*) or by a method of "shock lysis" (*1*). The unlysed cells and cell debris were sedimented at 10,000*g* to yield a supernatant fraction (S-10) which was used directly for studies of the incorporation of amino acid and was also used to prepare ribosomes and a ribosome-free supernatant solution (*1*). Ribosomes were separated into fractions, differing in sedimentation coefficients, by sucrose density-gradient centrifugation (see legend, Fig. 1). The components of the cell-free system for amino acid incorporation (Fig. 1), the counting procedures for determining radioactivity, and the processing of samples for determining incorporation of amino acid into protein have been described (*3*). Two preparations of polyU (*8*) were used with similar results.

The distribution of the C^{14}-phenylalanine that was incorporated on ribosomes by an S-10 preparation, both in the absence (endogenous reaction) and presence of polyU, was analyzed by the sucrose density-gradient technique (Fig. 1). The peak in ultraviolet (UV) absorption is designated 78*S* (a figure based on data from an analytical ultracentrifuge) and represents the marker ribosomes. The UV-absorbing material and radioactivity to the right of the 78*S* peak represent lighter components (Fig. 1, peak *a*). In the absence of polyU, radioactivity was associated with ribosomal fractions which had sedimentation coefficients greater than 100*S*, and there was no appreciable radioactivity in the 78*S* region (Fig. 1*A*). On the other hand, when incorporation occurred in the presence of polyU, radioactivity was distributed throughout the different classes of ribosomes. It should be noted that ribosomes with sedimentation coefficients of about 110*S* (Fig. 1*B*, corresponding to tube 15) had approximately twice as many count/min per unit of optical density as ribosomes with sedimentation coefficients of 78*S*.