Michigan surges and seiches has been given by Harris (*9*).

Conclusions

The theory of edge waves appears to explain most of the recorded effects of the storm surge of 6–7 July 1954. It seems reasonable to expect that relatively strong and fast-moving atmospheric disturbances, with large components parallel to the lake, have excited such edge waves in the past and will do so in the future. In cooperation with the U.S. Lake Survey, a program of instrumentation is under way to check this theory further and to provide empirical data for a more complete study of the Great Lakes surges. Edge-wave theory also appears to be applicable to the total reflection of deep-water waves near the beach as described by Isaacs *et al.* (*10*).

This study shows again that potentially dangerous waves may be expected at a shore station long after the passage of the generating disturbance in the air. It should be noted that the disastrous surge of 26 June 1954, which was described earlier (*1*) and was explained as originating from resonant coupling to gravity waves, does not permit explanation by the present mechanism, owing to the configuration of the lake bottom at the southern end of the lake and to the transverse path of the disturbance across the lake.

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Proteins and Calcium in Serums of Estrogen-Treated Roosters

Ole A. Schjeide and Marshall R. Urist

In 1935 Laskowski (*1*) discovered phosphoprotein, which he termed *serum vitellin,* in the plasma of laying hens. Riddle (*2*) in 1942 correlated the appearance of this protein with the elevated calcium that is seen in laying birds. Since that time, the protein-calcium system has received further elucidation. Several reports have appeared relating estrogen to elevated serum calcium (*3*). Other reports have related estrogen specifically to the appearance of phosphoprotein and other protein components in serum (*4*). Recently an interrelationship has been seen among all three entities, estrogen, phosphoprotein, and elevated calcium (*5*). In 1956, Clegg and coworkers demonstrated that injection of diethylstilbesterol into cockerels resulted in the introduction to the serum of phosphoprotein that complexed relatively large amounts of ionized calcium-45 that had been added to an *in vitro* system.

In connection with investigations on the comparative physiology of endosteal bone formation by Urist and McLean (*6*), studies utilizing the ultracentrifuge have been made of calcium-binding proteins in serums of roosters injected with massive doses of estrogen (*7*). With this material and the partition cell developed for the preparatory ultracentrifuge by Schjeide and Dickinson (*8*), further information has been obtained on calcium-binding components that are produced by avian species in response to estrogen (*9*).

Dr. Schjeide is on the staff of the Atomic Energy Project of the University of California at Los Angeles. He is also a member of the staff of the department of radiology in the School of Medicine. Dr. Urist is on the staff of the department of surgery in the School of Medicine.

Materials, Methods, and Results

A microsuspension of 125 milligrams of USP estrone (Ayerst) was injected intramuscularly into roosters. Beginning within 24 hours and rising nearly to a maximum within 5 days, there was a large increase in serum chylomicrons (*10*) and beta lipoproteins (*10*), nearly complete disappearance of alpha lipoproteins (*10*), and appearance of at least two new components (X_1 and X_2, as resolved by ultracentrifugation in sodium chloride (88 mg/ml, *p*H 6.0). In Fig. 1 are depicted schlieren patterns typical of control and estrone-injected roosters after removal (by floatation) of the chylomicrons and the beta lipoproteins. The concentration of albumin is practically the same in injected and control birds, despite a tenfold increase in phosphorus.

The X_1 and X_2 components display sedimentation rates of *S* 7.5 and *S* 15.0, respectively, when extrapolated to infinite dilution and corrected to 20°C (Fig. 2).

Analyses for calcium (*11*) in chylomicrons, beta lipoproteins, and alpha lipoproteins reveal that these bind less than 5 percent of the total calcium in the serum. Similar analyses on other components indicate that albumin and the denser globulins also bind comparatively small amounts of the serum calcium in estrone-injected birds. Within approximately 3 days after treatment, the calcium content of the serum in compartments 1, 2 and 3, 4 of the partition cell, after 9 hours of centrifugation in 10-percent NaCl at 70,000 times gravity, was as shown in Fig 3.

The elevated serum calcium seems to be mainly associated with the X_1 component. Correlations between the concentration of this species and protein-bound calcium are illustrated by the schlieren patterns shown in Fig. 4.

The X_1 species appears to be a phosphoprotein as shown by comparative studies of alkaline hydrolysis (after removal of lipids) on centrifugal fractions of serums from two different birds. One bird (*A* and *B*) has a very high level, and one bird (*C*) selected for comparison, has a relatively low level of protein phosphorus (Fig. 5).

The X_2 species is a very dense lipoprotein. Correlation of such data as total nitrogen (micro-Kjeldahl), schlieren patterns (ultracentrifuge), total lipid

(*12*), and constituent lipid (*13*), on whole serum minus chylomicrons, beta lipoproteins, and alpha lipoproteins, indicates the approximate composition (*14*) shown in Table 1.

The X_1 protein probably binds little lipid, for a strong correlation was observed between X_2 and the total lipid in fractions containing only albumin, X_1, and X_2.

It was found that component X_1 plus variable amounts of X_2 could be coprecipitated in uncontaminated form simply by dilution with distilled water. Most of the protein-bound calcium was released in the process. The precipitate could be resolubilized by addition of approximately 1-percent NaCl or a lesser amount of $CaCl_2$. When X_1 was separated by ultracentrifugation from X_2, it was soluble in distilled water. However, X_1, when combined with X_2 (water-soluble livetin) in distilled water, resulted in a precipitate composed of aggregates of the two types of molecules.

Previous investigators suggested that colloidal calcium phosphate may account for a large percentage of the non-ionized calcium in estrogenized serum (*15*). We account for nearly all the calcium in the serum as complexed with X_1, albumin, and lipoprotein, and this leaves relatively little for any such form as colloidal calcium phosphate.

Discussion and Conclusion

Although the relationship of the components X_1 and X_2 to the elevation of calcium in the plasma is of special interest, other valuable concepts have evolved from these findings. Some of these bear on the relationship between egg yolk vitellin and the $X_1 + X_2$ system of serum.

Vitellin (water-insoluble yolk protein) has been described in the older literature as a single substance. However, recent studies on frog and hen yolk vitellin (*16*) show that it is not a discrete molecule but actually consists of a system of at least two centrifugal and electrophoretic components. One of these is a phosphoprotein, and the other is a relatively dense lipoprotein. In the case of hen vitellin, the sedimentation rates of the components are very similar to

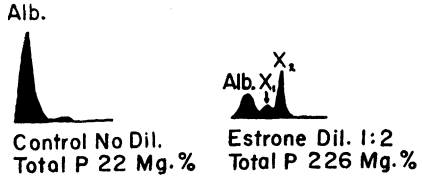

Fig. 1. Schlieren patterns of control rooster (left) and estrone-injected rooster (right). The areas of the peaks are proportional to concentration.

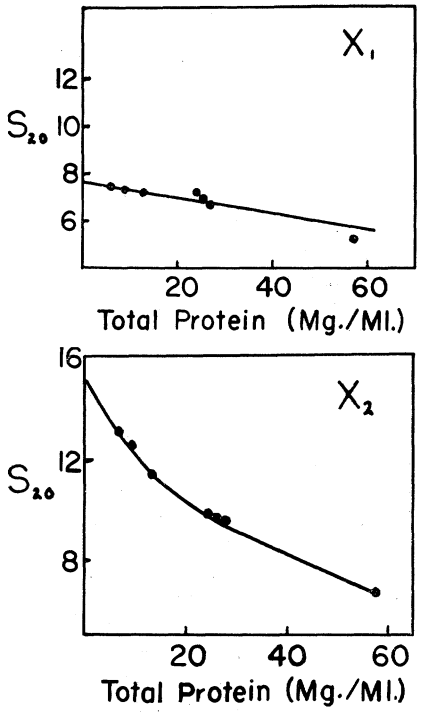

Fig. 2. Sedimentation rates of X_1 and X_2 components.

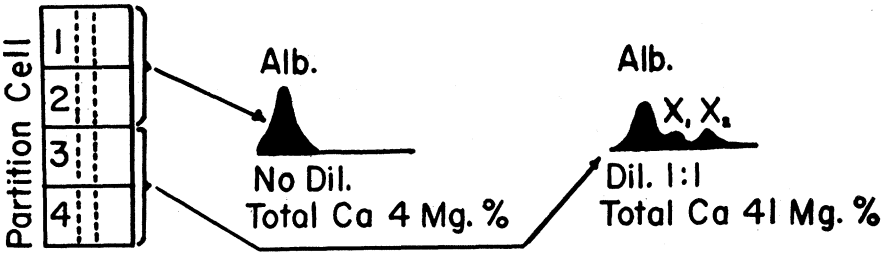

Fig. 3. Calcium content of serum present in the compartments of the partition cell. Chylomicrons and beta lipoproteins were removed by prior floatation. Note that globulins are present in very low concentration and can thus not be responsible for the elevated calcium.

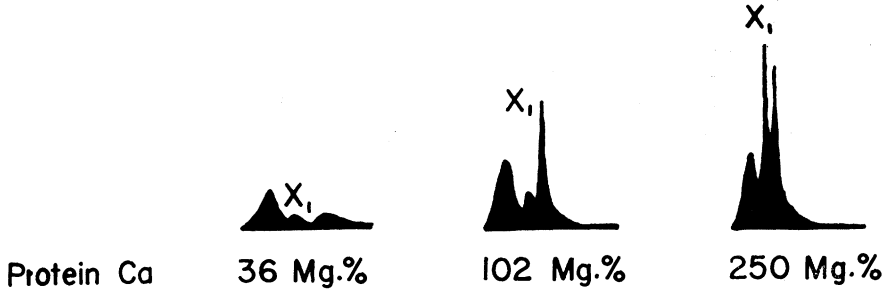

Fig. 4. Schlieren patterns in three different roosters from 3 to 5 days after treatment, showing the correlation between concentration of X_1 and protein-bound calcium. These materials are from compartments 3 and 4 of the partition cell after 9 to 16 hours of centrifugation at 70,000 times gravity. The protein identified with the X_1 peak in the case of the sample on the far right amounts to approximately 2100 milligram percent as concentrated in the fraction. Total serum calcium is approximately 130 milligram percent (*11*).

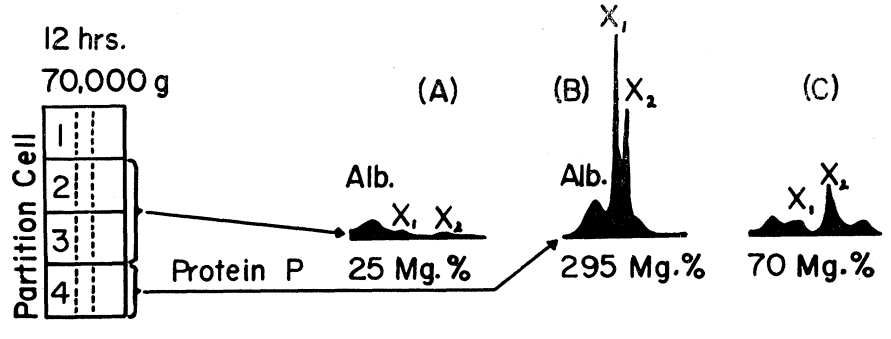

Fig. 5. Schlieren patterns showing the correlation between concentration of component X_1 and protein-bound phosphorus. The protein identified with X_1 peak in the case of sample *B* amounts to approximately 2600 milligram percent as concentrated in the fraction. Protein-bound calcium amounts to approximately 260 milligram percent.

Table 1. Approximate composition of component X_2. Each value was averaged from the results of three experiments.

Component	Amount (%)	Range
Protein	76.5	75.4–78.1
Lipid	23.5	21.9–24.6
Sterol ester	6.8*	6.4– 7.0
Triglyceride	18.8*	18.0–20.0
Sterol	6.2*	5.4– 7.0
Phospholipid	68.2*	66.0–69.6

* Percentage of the lipid component.

those of components X_1 and X_2 of the serum. In the egg yolk, these molecules are linked together as aggregates. Such aggregates are readily dissolved by addition of high concentrations of NaCl (5 percent) or low concentrations of $CaCl_2$ (less than 1 percent). Sodium chloride provides a solubilizing medium because of its high polarity; calcium probably acts by forming combinations with the phosphorus groups, rendering them inaccessible for further complexing. The dense lipoprotein, when it is encountered alone, seems to be identical with the water-soluble material in egg yolk which is commonly referred to as "livetin."

The demonstration of very similar two-component systems in both egg yolk and serum from estrogen-treated birds indicates the probability of a common origin of the protein in these biological materials. It is reasonable to suppose that most of the X_1 (phosphoprotein) and X_2 (lipoprotein) molecules in the serum are destined to be deposited in the egg yolk (phosphoprotein and lipoprotein usually occur in the same relative proportions in both systems). But this occurs as the protein is modified both in physical and in chemical form. The differences between the X_1 component of serum and the phosphoprotein of egg yolk appear to be that the X_1 component of serum is a soluble and discrete substance heavily complexed with calcium; the phosphoprotein of egg yolk is largely insoluble (in platelet or granule form) because it is complexed with X_2 lipoprotein (livetin). It is emphasized that components X_1 and X_2 are soluble in plasma only as long as sufficient dializable materials (probably calcium or similar ions) are present to keep them separated. Thus, in laying birds (and also in fish, amphibians, and reptiles, *17*) the elevated calcium-phosphoprotein-lipoprotein system of the serum apparently provides a method of transport and localization of storage nutrients (vitellin) for the embryo (*15*). The mechanism may not be unique to egg materials, but it may be fundamentally similar to other processes involving the deposition of insoluble structures in cells.

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10. For convenience, lipoproteins floating in a solution of density 1.003 are herein termed *chylomicrons;* complexes floating on solutions of density 1.063 are referred to as *beta* lipoproteins; and those floating on solutions of density 1.21 are labeled *alpha* lipoproteins.
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C. Neuberg, Biochemist

With the passing of Carl Neuberg on 30 May 1956 at the age of 78, biochemistry has lost one of its founders and leaders whose interpretation of metabolic events in terms of organic reactions created the pattern of inquiry into the mechanisms of intermediary metabolism.

Carl Neuberg was a complex personality, admired but feared by many, loved and understood by few. The strict social framework of imperial Germany, with its class consciousness and its military code of honor, provided a strange arena for the ingenuity, ambition, and drive of the young Carl Neuberg.

He studied in Berlin and Würzburg at a time when organic chemistry was dominated by such men as Emil Fischer, v. Baeyer, Wallach, Grignard; and physical chemistry by van't Hoff, Arrhenius, Nernst, and Ostwald; while biochemistry in Germany was represented by a sole chair occupied by Roehmann at the University of Breslau. Neuberg received his Ph.D. degree in 1900 with a thesis on the chemistry of glyceraldehyde carried out under the direction of A. Wohl. He was one of the last assistants of Virchow, the great cellular pathologist, and took his first steps in physiological chemistry in Salkowski's laboratory at the Agricultural College in Berlin.

Neuberg made his greatest scientific contributions to our understanding of fermentation and glycolysis. After Neubauer suggested pyruvic acid as the transitory intermediate in yeast fermentation, Neuberg's discovery of carboxylase provided a basis for the metabolic conversions of the keto acid. The cleavage of pyruvic acid into acetylphosphate and formic acid is a recent example of a general reaction first demonstrated by Neuberg in which α-keto acids are split into a fatty acid and formic acid. He introduced the method of the trapping of transitory intermediaries, enabling him to interpret correctly major phases in the mechanism of alcoholic fermentation and of the so-called glycerol fermentation. From these studies emerged the first indications for the mechanism of the cleavage of Harden-Young's hexosediphosphate into two subunits later iden-