a factor in egg formation. At present, the role of vitamins is being studied using adults that have been reared under aseptic conditions.

In these investigations on female mosquitoes, counts of the eggs produced provided a simple means of evaluating the nutritional requirements for egg development. Although the influence of diet on growth is perhaps more often measured in the immature animal, the nutrients required by the adult, in addition to those for maintenance and body repair, are of profound importance to the survival of the species through their effect on fecundity. The adult mosquito apparently requires little or no intake of protein for maintenance; hence, a limiting nutritional factor in reproduction is the availability of protein.

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Activation of Bacteriophage by Urea

Some strains of the bacteriophages T4 and T6 must first react with or be activated by certain amino acids and amino acid analogs, which are designated *cofactors*, before they can adsorb to their bacterial hosts. (*1*, *2*). The activation process is completely reversible. The most efficient of the known cofactors is L-tryptophan, which is demonstrably active at concentrations of $10^{-5}M$ (*3*). The nature of the interaction between these amino acids and the phage is unknown. It is the purpose of this communication to report that urea is capable of imparting adsorbability to a cofactor-requiring strain of T4 (*4*). The action of urea on proteins has been extensively studied (*5*), and the evidence from such studies leads to the conclusion that urea exerts its effect on protein by breaking weak secondary bonds within the macromolecule, changing thereby the rigid structure of the "native" to the looser configuration of the "denatured" protein.

Activation of cofactor-requiring strain T4.38 by urea has been demonstrated by means of the following procedure. Suspensions of the phage are incubated in urea solutions that are maintained at constant temperature. After various lengths of time, the phage are diluted out of the urea solutions into cofactor-free suspensions of 2×10^8 cells of *Escherichia coli*, strain B, per milliliter to allow adsorption of any phage activated by this treatment. After permitting sufficient time for adsorption to reach completion, the titer of adsorbed phage is then assayed by plating aliquots from the infected bacterial suspension on F agar (F agar is a cofactor-free synthetic substrate on which only those cofactor-requiring phage that have already adsorbed to bacteria before plating can form plaques, *1*). Treatment with urea, however, destroys the infectivity of the bacteriophage particles as well as activating their adsorbability to bacterial cells.

To determine the titer of phage that have survived the urea treatment, aliquots of the adsorption mixture are also plated on N agar. (N agar is a nutrient broth substrate on which both adsorbed and unadsorbed cofactor-requiring particles can form plaques.) The ratio of the assays on the two types of agar, F/N, is then the fraction of phages surviving the urea treatment which have adsorbed to bacteria under our standard conditions. This fraction is the outcome of two competitive processes: adsorption and the loss of urea-conferred adsorbability that takes place when the urea is diluted away. The adsorption of a urea-treated population of cofactor-requiring phages can also be demonstrated by use of a radiosulfur (S^{35}) labeled stock of T4.38. After incubation in urea solutions and dilution into bacterial suspensions, as mentioned, the fraction of the activated population adsorbed can also be assayed by counting the fraction of the total radioactivity that is sedimentable with the bacterial cells. The results of such an experiment, in which S^{35}-labeled phage were treated with nonbuffered 2.5M urea solutions at 0°C, are presented in Fig. 1.

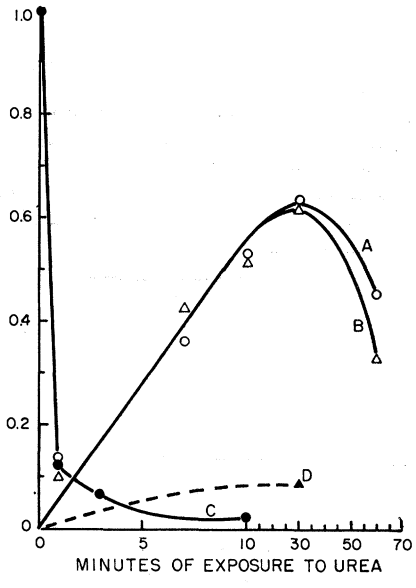

Fig. 1. *A*, fraction of S^{35} adsorbed to bacteria; *B*, fraction of survivors adsorbed to bacteria, F/N; *C*, fraction of survivors, N/N_0; *D*, fraction of S^{35} adsorbed to resistant bacterial strain, B/4.

Table 1. Activation rate constant, k, and killing rate constant, c, presented for different conditions of activation.

Conditions of activation				
Urea concn. (M)	pH	Temp. (°C)	k	c
2.5	6.5	4.5	0.0020	0.24
2.5	6.5	15	0.00017	0.02
2.5	6.5	37	0.0018	0.12
2.5	6.5	47	0.0030	0.58
2.5	6.5	0	0.14	0.92
3.0	6.5	0	0.82	3.7
2.0	6.5	0	0.0025	0.060
2.0	6.0	0	0.00027	0.0067
2.0	7.0	0	0.0075	0.30
2.0	8.0	0	0.020	1.20

First of all, it may be seen from curve *C* that this urea treatment rapidly inactivates the phage. From curve *B* it is seen that, initially, the longer the phage are incubated in the presence of urea, the greater the percentage of surviving phage that are adsorbed to the bacterial cells. Ultimately, this percentage decreases with increasing incubation time. By comparing curves *A* and *B*, it may be seen that the adsorbed fraction of the *surviving phage*, F/N, is equivalent to the fraction of the *total population* adsorbed to bacteria, as measured by the amount of adsorbed S^{35}. This equivalence, it is seen, holds over a wide range of survival values. Finally, from curve *D*, it is seen that urea-treated phage adsorb only to a slight extent to the resistant bacterial strain, B/4. Thus these phage have retained their specific adsorption characteristics. The results of the experiment shown in Fig. 1 and of other experiments not presented here show that, initially, the ratio F/N rises linearly with time, or $F/N = kt$. The fraction of survivors, N/N_0, can be shown to satisfy the relation, at the beginning of the inactivation, $\ln (N/N_0) = -ct$. In Table 1, the values of the rate constants, k and c, are presented that have been found on treatment of T4.38 with different urea concentrations at various pH and temperatures. It is apparent that both k and c are strongly dependent on the urea concentration, being proportional to approximately its twelfth power. Both of these constants, furthermore, are seen to be minimal at some temperature between 15° and 37°C. Finally, both k and c increase as the pH is raised from 6 to 8. An extensive similarity exists,

therefore, between the kinetics of killing and of activation of T4 bacteriophage by urea and the kinetics of protein denaturation by urea, for they exhibit entirely analogous concentration, temperature, and *p*H dependence.

We conclude that, through a range of values of *p*H and temperature, urea activates cofactor-requiring phage in a manner similar to its denaturing action —that is, by breaking critical hydrogen bonds in the protein of the phage adsorption organ to produce a new configuration, which is able to form an attachment to the bacterial surface. It seems reasonable to postulate further that tryptophan-like cofactors exert their influence in a similar manner.

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Chenodeoxycholic Acid in Human Blood Serum

For many years chenodeoxycholic acid (3,7-dihydroxycholanic acid) was thought to be confined largely to the bile of fowls and to occur only as a minor component of human bile. Recent studies (*1*) have shown, however, that this bile acid is a prominent constituent of human bile and that it is also one of the principal bile acids in human blood serum (*2*). After considering the numerous methods for measuring blood cholates with their highly variable results, which ranged from none at all to values exceeding 100 mg percent, Sobotka (*3*) concluded that reliable procedures must be preceded by a reasonable amount of evidence that the bile acid being quantitated actually exists in the serum. The present study is designed to furnish some of this necessary evidence and thus to provide a basis for further quantitative studies of the several bile acids in human serum.

An extract of serum was prepared according to methods that have previously been described (*4*). The procedure employed was as follows: serum was removed from freshly clotted blood, and 25 ml was added dropwise to a mixture of 250 ml of ethanol and 15 ml of a saturated aqueous solution of barium hydroxide that contained 1 g per 100 ml of barium acetate. The mixture was heated 10 minutes in a boiling water bath, and the precipitated proteins were removed by filtering through a medium sintered glass funnel. The filtrate was taken to dryness under reduced pressure at 40° to 45°C. The dry residue was stirred with 100 ml of ethyl acetate to which 2.5 ml of a 3.3-percent solution of anhydrous calcium oxide in ethyl acetate had been added. The mixture was heated in a boiling water bath for 2 minutes; it was then centrifuged, and the supernatant was decanted. The ethyl acetate wash was repeated twice more in the same manner, except that the calcium oxide reagent was omitted. The residue was then dissolved in 10 ml of 7*N* sodium hydroxide and autoclaved for 3 hours at 140°C. After hydrolysis, the alkaline solution was made up to a volume of 50 ml with water, acidified with concentrated hydrochloric acid to a blue reaction with congo red paper (*p*H less than 3), and extracted four times with 50-ml portions of chloroform. The chloroform extract was concentrated to dryness under reduced pressure, and the residue was taken up in acetone and adjusted to a volume of 1 ml.

Paper chromatography was carried out as described by Sjovall (*5*) by applying 0.2 ml of the acetone solution to the paper. Solutions of cholic acid (3,7,12-trihydroxycholanic acid), deoxycholic acid (3,12-dihydroxycholanic acid) and chenodeoxycholic acid in acetone were also applied in amounts ranging from 1 to 40 μg to serve as controls. Antimony trichloride reagent was used for identification as previously described (*6*). In a system using 20-percent isopropyl ether in heptane, descending for 18 hours, a well-defined spot could be seen that moved at the same rate as the known chenodeoxycholic acid and that also had a purple fluorescence identical with that of the chenodeoxycholic acid control.

By this means chenodeoxycholic acid has been observed repeatedly in both normal and icteric serums. Cholic acid and deoxycholic acid have also been regularly found by employing 60-percent isopropyl ether in heptane. For normal serum it is sometimes necessary to apply larger amounts of the acetone solution in order to detect the minute amounts of bile acid present. If hydrolysis is not done, the bile acids occur as conjugates of glycine and taurine, and a different solvent system is required for chromatography; no free bile acids have been observed in human serum thus far.

In these qualitative studies, no attempt has been made to measure the amounts of bile acids present beyond comparing the size and fluorescent intensity of spots from serum with that of the known amounts of acids that were applied as controls. By this process, it was observed that cholic acid and chenodeoxycholic acid occur in approximately equal amounts, the former usually predominating in normal serum. Deoxycholic acid occurred in much smaller amounts. Wysocki and associates (*7*) have noted that an extract of serum dissolved in a mixture of sulfuric and acetic acids had a total absorbancy at 3100 A that exceeded the values contributed by deoxycholic acid and cholic acid at this wavelength; they concluded that the difference was due to chenodeoxycholic acid. This conclusion is supported by our findings. Their observation that the concentration of chenodeoxycholic acid is approximately 7 times that of cholic acid is probably accounted for by the differences in the methods used (*8, 9*).

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Lipid Levels in Migrating Birds

Although it is generally known that the body fat content of migratory birds increases greatly at the time of migration, few actual measurements of total lipids have been made. In October 1954, we obtained a sample of birds representing many species that had been killed by flying into obstacles near airport ceilometers. On the nights of 7 and 8 October a large concentration of birds was evidently "riding a cold front" southward when peculiar weather conditions brought about accidents at a number of airports (*1*). Specimens frozen shortly after death were obtained for us by D. W. Johnston of Mercer University, Macon, Ga., and by Ivan Tomkins at Savannah, Ga. This material has enabled us to compare lipid deposition of birds that were literally "snatched from the air" during migration with previous data that were obtained from premigrant, nonmigrant, and experimental birds. In