ized procedure described by Itano (*10*). In this procedure, a 50-mg sample of a hemoglobin or hemoglobin mixture is salted out at 25°C in 10 ml of aqueous solvent that is 2.58*M* in potassium phosphate buffer of *p*H 6.8 and contains 100 mg of sodium dithionite ($Na_2S_2O_4$). The concentration of hemoglobin remaining in solution is then measured. Four determinations on one of the AJ samples yielded a value of 2.00 ± 0.24 g/lit, which is significantly higher than the solubility, 1.39 ± 0.15 g/lit, found for amorphous ferrohemoglobin A under the same experimental conditions (*10*).

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6. In a recent communication A. C. Allison [*Science* 122, 640 (1955)] assigned the letter J to the hemoglobin reported by J. D. Battle and L. Lewis [*J. Lab. Clin. Med.* 44, 764 (1954)] and the letter K to the hemoglobin reported in the present work. This assignment, which is the reverse of that previously agreed upon by the workers concerned (see Itano, *1*) resulted from a misunderstanding in the course of conversations and correspondence between Allison and us. Allison has agreed to our inserting this correction to his communication in the present paper.
7. R. W. Smith and C. L. Conley, *Bull. Johns Hopkins Hosp.* 93, 44 (1953).
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Role of Diet in Egg Development by Mosquitoes (Aedes aegypti)

Because of their importance as vectors of disease, the yellow fever and malaria mosquitoes have been subjected to intensive investigation for more than 50 years. Nevertheless, the nutritional requirements for the development of eggs in *Aedes aegypti* and *Anopheles quadrimaculatus* have never been determined. Until recently, it was generally assumed that the blood-sucking species of mosquitoes required a blood meal to mature their ova. The only studies on adult mosquito nutrition to date have been with blood fractions (*1*) or with supplements to blood fractions (*2*), but these investigations have not provided sufficient information on which to base any conclusions about the role and relative importance of such blood constituents as amino acids, lipids, carbohydrates, minerals, and vitamins. A significant difference in the number of eggs produced by mosquitoes that take blood from different host animals has been observed by several investigators, but the nutritional factors essential to the development of eggs must be known before the effect of diet on fecundity can be explained.

In a preliminary note from this laboratory, Lea *et al.* (*3*) reported that both *Ae. aegypti* and *An. quadrimaculatus* would ingest a skim milk and honey solution from a saturated cotton pad and would subsequently develop and lay viable eggs. In the present study (*4*), numerous substances in sugar solution and on saturated pads were fed to cages of 200 fertile female *Aedes* for 16 days. Although the test food was always available to the mosquitoes, no attempt was made to control the amount of food ingested or the number of insects feeding at any time. Of the foods tested, only certain proteins or their enzymatic hydrolyzates were found to stimulate egg production. Daily counts of the eggs laid over a 16-day period have been totaled for several of these foods (Table 1). Oviposition by *An. quadrimaculatus* followed the feeding of either egg albumin or proteose-peptone, the only foods tested on this species other than milk.

Although both species of mosquitoes will remain alive and vigorous for several months on a sugar solution alone, neither species has ever been known to mature eggs on a sugar diet. Therefore, it was evident that in the protein-sugar mixtures tested, protein was a major nutritional factor required for egg development and consequently for reproduction by the female mosquito.

The feeding tests were extended to include known mixtures of purified amino acids, thus affording a more accurate means of evaluating the importance of each amino acid in the diet. A medium containing 18 amino acids, dextrose and levulose, and a salt mixture was formulated, which, when fed for 14 days to cages of 400 female *Aedes,* resulted in oviposition of viable eggs. Another mixture of 12 acids (Table 2, medium A) was found to be as effective as the mixture of 18 acids. From medium A, each amino acid was omitted singly, and the effect on egg production was observed in the egg counts. Of the 12 acids, there were eight (arginine through valine) which, when omitted, made the medium inadequate for the development of any eggs. The omission of histidine or methionine so limited the rate of ovarian growth that only a few eggs were laid, while the omission of cystine or glutamic acid reduced oviposition to a lesser degree. Although some eggs were laid on a mixture of the eight acids alone, the best results have been obtained with a medium containing all 12 amino acids.

Tests were also made in which the concentration of each of the 12 acids was varied. The optimum quantity of each acid was then used to establish a new medium (Table 2, medium B) that enabled the mosquitoes to lay an average of 14,000 eggs in 14 days. This was twice the number laid on medium A and indicated the importance of proper balance among the amino acids in the mixture. In addition, some preliminary tests of other factors in blood showed that lipids are dispensable but that minerals may be

Table 1. Egg production from test foods. The values represent egg production after 16 days from 200 *Ae. aegypti* females. The liquid foods contained 90 ml plus 10 ml of honey; the dry foods contained 10 g plus 10 ml of honey diluted to 100 ml with water.

Food	Eggs (No.)
Citrated (hemolyzed) beef blood	15,905
Fresh skim milk	3,072
Powdered egg albumin	9,408
Proteose-peptone	2,815
Enzymatic digest of soybean meal	1,092
Enzymatic digest of yeast	2,416
Enzymatic digest of casein	7,409
Enzymatic digest of lactalbumin	2,733

Table 2. Amino acid composition of test diets. Each medium also contained 5 g dextrose, 5 g levulose, 0.15 g of a salt mixture, and 100 ml of water.

Amino acid	Composition (g/100 ml)	
	Medium A	Medium B
L-Arginine	0.5	0.38
DL-Isoleucine	1.0	0.50
L-Leucine	1.0	0.75
L-Lysine	0.9	0.75
DL-Phenylalanine	0.7	1.20
DL-Threonine	0.8	0.30
L-Tryptophan	0.4	0.30
DL-Valine	1.0	1.00
L-Histidine	0.7	0.15
L-Methionine	0.2	0.15
L-Cystine	0.2	0.15
L-Glutamic acid	1.0	1.00

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.

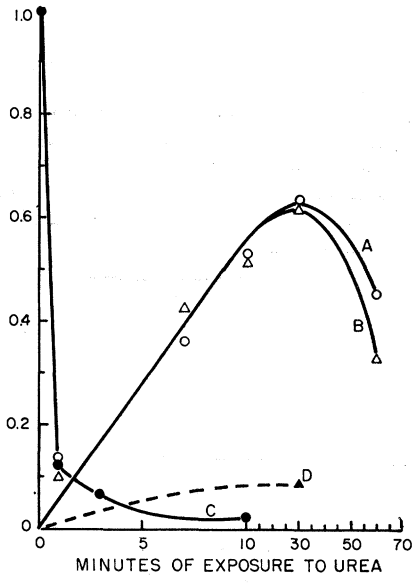

Fig. 1. *A*, fraction of S^{35} adsorbed to bacteria; *B*, fraction of survivors adsorbed to bacteria, F/N; *C*, fraction of survivors, N/N_0; *D*, fraction of S^{35} adsorbed to resistant bacterial strain, B/4.

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,