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.

G. H. SATO*
*California Institute of Technology, Pasadena*

**References and Notes**

1. T. F. Anderson, *J. Cellular Comp. Physiol.* 25, 17 (1945).
2. ———, *Cold Spring Harbor Symposia Quant. Biol.* 11, 1 (1946).
3. G. S. Stent and E. L. Wollman, *Biochim. et Biophys. Acta* 6, 292 (1950).
4. G. H. Sato, thesis, California Inst. of Technology, 1956.
5. H. Neurath and K. Bailey, *The Proteins* (Academic Press, New York, 1950), chapt. 9.

* Present address: Virus Laboratory, University of California, Berkeley.

21 November 1955

## 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*).

J. B. CAREY, JR.
*Department of Medicine, University of Minnesota Medical School, Minneapolis*

**References and Notes**

1. I. D. P. Wootton, *Biochem. J. London* 55, 292 (1953); B. Isaksson, *Acta Soc. Med. Upsaliensis* 59, 307 (1954).
2. J. B. Carey, Jr., *J. Lab. Clin. Med.* 46, 802 (1955).
3. H. Sobotka, *Physiological Chemistry of the Bile* (Williams and Wilkins, Baltimore, 1937), p. 112.
4. B. Josephson, *Biochem. J. London* 29, 1519 (1935); H. Minibeck, *Biochem. Z.* 297, 29 (1938); J. L. Irvin, C. G. Johnston, J. Kapola, *J. Biol. Chem.* 153, 439 (1944).
5. J. Sjovall, *Acta Chem. Scand.* 8, 339 (1954).
6. J. B. Carey, Jr., and H. S. Bloch, *J. Lab. Clin. Med.* 44, 486 (1954).
7. A. P. Wysocki, O. V. Portman, G. V. Mann, *Arch. Biochem. and Biophys.* 59, 213 (1955).
8. This investigation was supported by a research grant, A-713, from the National Institute of Arthritis and Metabolic Disease, National Institutes of Health, U.S. Public Health Service.
9. The advice of C. J. Watson and the technical assistance of J. Figen is gratefully acknowledged.

28 October 1955

## 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

this paper we are concerned only with the order of magnitude of lipid depositions; we have left further details of the nature and formation of the remarkable "migratory fat" for subsequent publications.

Specimens while still frozen were ground up in a chilled food chopper and then dehydrated and partially extracted in cold alcohol and ether, 3:1. The solid residue was extracted in a Soxhlet (for large species) or Bailey-Walker (for small species) extractor using petroleum ether and chloroform, 5:1. The two extracts were then evaporated to sticky dryness and reextracted together with petroleum ether, thus leaving behind the small amounts of alcohol-soluble proteins and other impurities. The total extracts were evaporated over a steam bath and dried to constant weight in a vacuum desiccator. The nonfat dry weight was also obtained. The results are given in Table 1.

Total lipids are expressed as a percentage of both total wet weight and total dry weight. All the October "ceilometer" birds proved to be quite fat, with little evident difference between sexes or between first year (easily identified by incompletely ossified skulls) and older birds. The first seven species listed in Table 1 migrate south of the United States for the winter and in some cases, at least, nonstop for 500 miles or more over the Gulf of Mexico. Individuals of these species were quite uniformly fat as shown by the low coefficients of variation. On the other hand, the yellowthroats that winter partly in the southern United States and partly in the West Indies and Mexico were not only less fat than the species that travel farther but also showed greater individual variation.

In Table 2 average lipid levels of the migrating birds are compared with those of premigrant, postmigrant, and experimental birds of species of various migratory status. These data indicate that the highest levels occur just prior to or during migration in species that winter in Central or South America. In this group of "overseas migrants," a third of the wet weight or nearly two-thirds of the dry weight may be lipids, most of it in the form of huge subcutaneous and interperitoneal fat deposits. By contrast, nonmigratory species—that is, "permanent residents"—and migratory species during periods of nonmigratory activity proved to carry not more than 6 or 7 percent fat. The white-throated sparrow, which does not migrate south of the continental United States, is intermediate between these two extremes. The maximum lipid deposition that could be induced experimentally by long photoperiods in captive individuals of this latter species was almost exactly the same as observed in wild birds that had been captured a few days prior to northward migration in the spring.

It is interesting and perhaps significant that all but one of the major parts and organs of the body increase in lipid content just before migration; the exception is the heart, which shows no increase in fat content whatsoever (*2*). Thus, fuel is stored in all parts of the body, but unlike the condition in obese human beings, the heart is not hampered by an excess of fat.

The remarkable fat deposition in ruby-throated hummingbirds is of special interest, for it is thought that these tiny birds make a long overwater flight to Central America. Pearson (*3*) has measured the rate of metabolism of flying hummingbirds and has estimated the amount of energy needed for long flights. Having no data on fat deposition at the

Table 1. Lipid levels in a sample of birds killed while in actual migratory flight over Georgia, 7–8 Oct. 1954.

| Species | No. | Avg. wet wt. (g) | Total lipids as percentage of wet and dry wt. | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Wet wt. | | | Dry wt. | | |
| | | | Avg. | Extremes | V* | Avg. | Extremes | V* |
| Black-throated blue warbler (*Dendroica caerulescens*) | 16 | 10.6 | 27.6 | 21.6–35.7 | 14.3 | 55.5 | 45.6–61.9 | 9.0 |
| Ovenbird (*Seiurus aurocapillus*) | 16 | 21.7 | 29.7 | 27.4–34.1 | 8.0 | 56.8 | 53.5–62.2 | 5.6 |
| Bay-breasted warbler (*Dendroica castanea*) | 4 | 14.7 | 31.0 | 25.7–35.5 | | 60.6 | 52.3–64.3 | |
| Summer tanager (*Piranga rubra*) | 6 | 39.4 | 37.2 | 32.9–41.8 | 8.6 | 66.02 | 62.6–70.3 | 3.9 |
| Scarlet tanager (*Piranga erythromelas*) | 3 | 37.8 | 33.9 | 30.1–37.0 | | 63.0 | 58.4–66.9 | |
| Philadelphia vireo (*Vireo philadelphicus*) | 6 | 13.4 | 26.2 | 21.2–32.1 | 16.2 | 56.6 | 49.5–63.9 | 10.3 |
| Red-eyed vireo (*Vireo olivaceus*) | 4 | 20.6 | 28.5 | 22.5–32.5 | | 58.8 | 51.5–65.8 | |
| Yellowthroat (*Geothlypis trichas*) | 13 | 10.9 | 22.2 | 11.3–31.0 | 32.2 | 48.0 | 30.2–62.8 | 22.7 |

* V is the coefficient of variation.

Table 2. Comparison of lipid levels in birds of various migratory status.

| Species | No. of individuals | Total lipids in percentage of wet wt. (g/100 g) | |
|---|---|---|---|
| | | Avg. | Extremes |
| *Overseas or long-distance migrants* | | | |
| Seven species of warblers, vireos, and tanagers in actual southward migration over Georgia; data from Table 1 | 55 | 29.8 | 21.2–41.8 |
| Ruby-throated hummingbird (*Archilochus colubris*), premigration deposition just prior to southward migration (*4*) | 4 | 43.0 | 40.6–45.9 |
| Ruby-throated hummingbird during summer nonmigratory period (June and July) | 3 | 13.4 | 11.1–15.1 |
| *Continental Migrant, the white-throated sparrow* (Zonotrichia albicollis)* | | | |
| Premigration deposition in Georgia just prior to northward migration in April (*2*) | 19 | 16.7 | 9.7–24.8 |
| Maximum deposition obtained experimentally by increased photoperiods in mid-winter (*5*) | 22 | 15.7 | 11.8–24.5 |
| Postmigration lipid levels after birds had reached Georgia wintering grounds in October and November (*2*) | 23 | 6.8 | 4.3–12.8 |
| *Nonmigrants (permanent residents)* | | | |
| English sparrows (*Passer domesticus*) and Carolina wrens (*Thryothorus ludovicianus*) in Georgia in October | 17 | 3.5 | 2.1– 6.7 |

* *Z. albicollis* breeds in the northern United States and Canada and winters in the southern United States.

time of his publication, he estimated (on the basis of weight differences) that about 1 g of fat would be the maximum available for migration. This amount would provide energy for a flight of only about 385 miles, not enough to get the bird across the Gulf of Mexico. However, the four premigrant hummingbirds in Table 2 actually had an average fat content of 2.1 g, as compared with about 0.4 g for summer individuals. Using Pearson's metabolic rate figures, 2.1 g of fat would allow a flight of 800 miles. Even if 0.4 g (the summer level) were unavailable for flight, the remaining 1.7 g would last for 655 miles, more than enough to span the Gulf.

EUGENE P. ODUM
CLYDE E. CONNELL
*Department of Zoology, University of Georgia, Athens*

**References and Notes**

1. D. W. Johnston, *Oriole, Georgia Ornithol. Soc.* 20, 17 (1955).
2. E. P. Odum and J. D. Perkinson, *Physiol. Zool.* 24, 216 (1951).
3. O. P. Pearson, *Condor* 52, 145 (1950).
4. One of the premigrant hummingbirds was obtained on the Gulf coast of Florida 4 Nov. by Herbert Stoddard, and three were captured near Augusta, Ga., on 26 Sept. by Robert Norris.
5. Data from E. P. Odum and J. C. Major, *Condor*, in press.

9 November 1955

## Effect of Foliar Sprays of Maleic Hydrazide on Photosynthesis

Maleic hydrazide (1,2-dihydropyridazine-3,6-dione) was found to decrease the number and increase the size of chloroplasts in lettuce (*1*). We are now able to report that this compound produces similar effects in other plants, and that there is an accompanying effect on photosynthesis.

In the experiments reported here (*2*) two concentrations of maleic hydrazide (MH) were used, 0.375 g/lit and 3.0 g/lit (*3*). Swiss chard was raised from seed in the greenhouse. In the cotyledon stage, or after the formation of two to three leaves, the seedlings were sprayed with the MH solution. Leaves that developed subsequent to the treatment were used for photosynthesis studies (*4*). Tobacco plants were sprayed in a similar manner, except that the plants had five to six leaves at the time of treatment.

Table 1. Number and size of chloroplasts in normal swiss chard leaves and in leaves treated with maleic hydrazide.

| Chloroplasts | Normal | Treated | $LSD_{.01}$* |
|---|---|---|---|
| No. per cell | | | |
| Palisade | 73.0 | 42.5 | 19.6 |
| Spongy parenchyma | 81.3 | 57.1 | 21.2 |
| Diameter (μ) | 5.74 | 7.04 | 0.464 |

* Least significant differences at the 0.01 level of probability.

Table 2. Rates of photosynthesis and respiration and chlorophyll concentrations of normal tobacco leaves and leaves treated with maleic hydrazide.

| Item | Normal | MH-treated (0.375 g/lit) | MH-treated (3.0 g/lit) |
|---|---|---|---|
| Photosynthesis (μlit $O_2$/$cm^2$ hr) | 9.17 | 12.08 ($LSD_{.01}$ = 2.14) | 14.42 ($LSD_{.01}$ = 1.72) |
| (μlit $O_2$/mg of chlorophyll per hour) | 1128 | 1928 ($LSD_{.01}$ = 438) | 1545 ($LSD_{.01}$ = 441) ($LSD_{.05}$ = 307) |
| Respiration (μlit $O_2$/$cm^2$ hr) | 1.93 | 1.74 | 1.04 |
| Chlorophyll (mg/$cm^2$) | 0.00813 | 0.00636 | 0.00933 |

Chloroplast structure in normal and treated leaves was studied microscopically. Disks of leaf tissue were macerated in a mixture of 1*N* hydrochloric acid and 50 percent ethyl alcohol. Chloroplast size was determined by means of an ocular micrometer, and the number of chloroplasts per cell was determined by counting the chloroplasts in a large number of cells.

Oxygen evolution by leaf disks was measured manometrically at 25°C. Disks were punched with a cork borer, and ordinarily 10 $cm^2$ was suspended in 0.1*M* $KHCO_3$ in standard Warburg flasks. Two Lumiline tubes, supported in the bath 1 cm below the vessels, provided a light intensity of 200 ft-ca at the level of the leaf tissue. Respiration measurements made in the dark were used to correct the oxygen evolution. Dry weights of the tissue used in the vessels, or of separate aliquots, were determined by oven-drying to constant weight. Chlorophyll was determined with the Beckman DU spectrophotometer, using the method of Arnon (*5*).

Leaves that developed subsequent to treatment were noticeably darker green than normal leaves. The alterations in chloroplast morphology found by Callaghan (*1*) in lettuce were found also in swiss chard and tobacco. Representative data from one treatment are presented in Table 1. Tobacco chloroplasts are somewhat smaller than those of swiss chard, but the effect of MH is about the same as that shown in Table 1.

Rates of photosynthesis are significantly increased by pretreatment with MH, as is shown in Table 2. At the lower concentration of MH there is relatively little effect on dry weight, respiration rate, and chlorophyll concentration. The increase in the rate of photosynthesis, however, is quite spectacular. It is to this increase that we attach the most significance.

At the higher concentration, the depression of respiration and the somewhat higher chlorophyll concentration account for a part of the apparent increase in photosynthetic rate. In leaf disks from plants treated with the higher concentration of MH, the dry weights per unit area were quite variable and depended on the recent history of the plants. Thus, chlorophyll or area is a better basis upon which to compare photosynthetic rates.

Swiss chard yielded values comparable to those shown for tobacco.

Maleic hydrazide has many effects on higher plants, most of which result from changes in the developmental sequence in the terminal meristem (*6*). At the lower concentration used here, however, there was little obvious morphological change in size or shape of leaves. The measured increase in photosynthetic rate seemed to be a modification of the physiology of the individual leaf cells. At present it is impossible to tell how the observed changes are brought about. The great change in rate per unit of chlorophyll suggests an alteration in the photochemical mechanism of photosynthesis. The low light intensity used here contributes to this suggestion. It is possible that this technique will provide an approach to the problem of energy transfer in photosynthesis.

The increase in photosynthetic rate has practical implications. Indeed, Mikkelsen *et al.* (*7*) found an increase in yield of sucrose from MH-treated sugar beets. However, they suggested only casually that there might be a direct effect on photosynthesis. In other experiments (*8*), they found increased sucrose concentration but no increase in yield.

JOHN J. CALLAGHAN*
RICHARD W. VAN NORMAN†
*Department of Botany and Plant Pathology, Pennsylvania State University, University Park*

**References and Notes**

1. John J. Callaghan, M.S. thesis, Cork University, Cork, Ireland (1953).
2. Contribution No. 202 from the department of botany and plant pathology, Pennsylvania State University, University Park.
3. Concentrations of the active ingredient in a solution of the diethanolamine salt of MH. Alconox detergent was added as a spreading agent.