antibody present to itself and to RI/5/57.

Summary. Six strains of influenza A virus were isolated in New York during the 1957 pandemic. Close antigenic relationship was found among these strains and 2 Far East isolates, and slight antigenic relationship to older prototype strains was also demonstrated. One of the local isolates, RI/5/57, was found to be a very sensitive indicator of the presence of hemagglutination-inhibiting and neutralizing antibodies against the 1957 strains of influenza A virus in human serum. Antibodies to the RI/5/57 strain were demonstrated in patients of various ages obtained from 1940 to 1957. The sensitivity of the 6 New York strains to non-specific inhibitors of hemagglutination varied from apparent insensitivity in 3 strains to extreme sensitivity in 2.

1. Jensen, K. E., Hogan, R. B., *Pub. Health Rep.*, 1958, v73, 140.

2. Meyer, H. M., Jr., Hilleman, M. R., Miesse, M. L., Crawford, I. P., Bankhead, A. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 609.

3. Jensen, K. E., *J.A.M.A.*, 1957, v164, 2025.

4. Mulder, J., *Lancet*, 1957, v2, 334.

5. Davenport, F. M., *Pub. Health Rep.*, 1958, v73, 133.

6. Hilleman, M. R., Flatley, F. J., Anderson, S. A., Leucking, M. L., Levinson, D. J., *J.A.M.A.*, 1958, v166, 1134.

7. Boger, W. P., Liu, O. C., *ibid.*, 1957, v165, 1687.

8. Tyrrell, D. A. J., Horsfall, F. L., Jr., *J. Immunol.*, 1952, v69, 563.

9. Hilleman, M. R., Horsfall, F. L., Jr., *ibid.*, 1952, v69, 343.

10. Archetti, I., Horsfall, F. L., Jr., *J. Exp. Med.*, 1950, v92, 441.

11. Jensen, K. E., Francis, T., Jr., *ibid.*, 1953, v98, 619.

12. Fiset, P., Depoux, R., *Bull. World Hlth. Org.*, 1954, v11, 987.

13. Hirst, G. K., *J. Exp. Med.*, 1947, v86, 357.

14. Sugg, J. Y., *J. Bact.*, 1949, v58, 399.

15. Jensen, K. E., Minuse, E., Francis, T., Jr., *J. Immunol.*, 1957, v78, 356.

16. Tamm, I., Ginsberg, H. S., Horsfall, F. L., Jr., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 94.

17. Isaacs, A., Gledhill, A. W., Andrewes, C. H., *Bull. World Hlth. Org.*, 1952, v6, 287.

18. Isaacs, A., *Lancet*, 1953, v1, 676.

Received April 28, 1958. P.S.E.B.M., 1958, v98.

Studies on Copper Metabolism. XXV. Relationship Between Serum and Liver Copper.* (24093)

H. DEMPSEY,† G. E. CARTWRIGHT AND M. M. WINTROBE

Department of Medicine, University of Utah College of Medicine, Salt Lake City

In a previous publication from this laboratory(1), 2 infants with hypochromic anemia, hypocupremia, hypoferremia and hypoalbuminemia were described. The parenteral administration of iron was followed by prompt hematologic response and by appreciable increase in serum albumin, but not by an increase in serum copper. The hypocupremia was alleviated only by administration of copper. It was suggested that hypocupremia was secondary to a deficiency of copper which was great enough to produce hypocupremia but which was not sufficiently severe to be significant in the pathogenesis of anemia. Little is known concerning the interrelationship between dietary, serum and liver copper. It has been shown in both swine(2) and rats(3) fed diets low in copper that hypocupremia precedes development of anemia. The extent to which serum copper concentrations reflect tissue concentrations of copper in animals fed diets containing low, normal and increased amounts of copper is unknown.

This paper presents observations on correlation between serum and liver copper in rats fed diets containing low, normal and increased amounts of this element. Data on blood he-

* This investigation was supported by research grant (C-2231) from Nat. Cancer Inst., N.I.H., U.S.P.H.S.

† Medical Research Fellow, Canadian National Research Council.

TABLE I. Observations on Hemoglobin, Serum Copper and Serum Iron, Liver Copper and Total Serum Proteins.

Group	Day	Hemoglobin, g %	Serum copper, μg %		Wt, g	Copper, μg/g†		Serum iron, μg %‡	Total protein, g %‡
Normal copper	0		138	6.9*	8.98	3.53	.03*	161	6.8
	10		142	10.6	6.15	3.57	.23	278	
	20		135	6.2	6.58	3.73	.23	207	
	40	15.3	121	4.7	6.07	4.10	.05	222	
	60	15.5	133	6.4	5.54	3.84	.04	238	
Low copper	5		65	5.0	8.98	3.26	.07		
	10		58	3.6	7.04	2.67	.15	233	6.4
	20		44	6.6	6.80	1.58	.15	210	5.9
	40	15.5	23	4.2	6.38	2.31	.15	187	5.2
	60	14.6	24	4.4	6.14	2.08	.12	130	6.3
High Copper	0		93	2.9	6.30	4.84	.35	147	
	10		119	3.9	4.78	3.63	.35	218	
	20		132	2.7	5.44	6.38	.62	233	
	40		149	5.8	4.98	8.95	1.11	281	
	60		184	8.7	4.71	12.16	1.00	182	

* The figures refer to the mean ± stand. error of groups of 10 rats. † μg/g of wet tissue.
‡ Pooled samples.

moglobin, total serum proteins and serum iron are also reported.

Methods. One hundred and fifty male, Sprague-Dawley rats, 150 to 200 g in weight were used. The basal diet consisted of evaporated milk,‡ diluted 1:1 with demineralized water. 25 mg of ferric chloride,§ spectroscopically-free of copper, was added to 1 l of the basal diet. Since each animal consumed about 50 ml of the basal diet daily, each received 1.25 mg of iron/day. The diet fed to animals in the "normal-copper" group was supplemented with copper acetate in such an amount that each animal received 40 μg of copper daily. The diet fed animals in the "high-copper" group was supplemented with copper acetate in such an amount that each animal consumed 1.6 mg of copper daily. Ten animals in the "normal-copper" group were sacrificed on days 0, 10, 20, 40 and 60. The "low-copper" group was started on the experiment at the same time as the "normal-copper" group and 10 animals were sacrificed on days 5, 10, 20, 40 and 60. The "high-copper" group was studied at a later date and 10 rats were sacrificed on days 0, 10, 20, 40 and 60. All animals were anesthetized lightly with ether and exsanguinated *via* the abdominal aorta. The livers were removed, rinsed with copper-free water, weighed and stored in the frozen state. Methods for determination of serum copper(4), serum iron(5) and tissue copper(6) have been published previously. Total serum protein estimations were performed by the method of Weichselbaum(7). Hemoglobin was determined by the cyanmethemoglobin method(8).

Results. The data are presented in Table I and in Fig. 1. Concentration of copper in

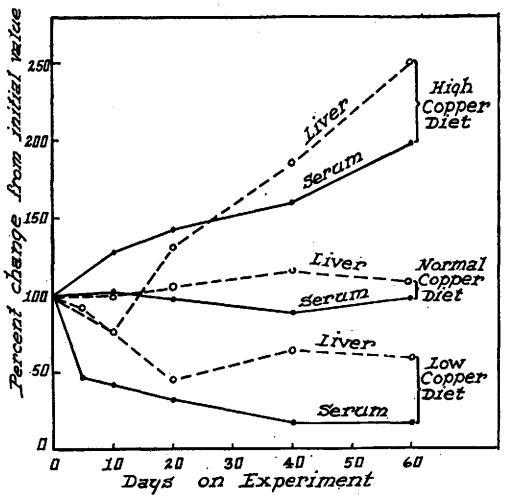

FIG. 1. Influence of dietary copper on concentration of copper in serum and liver. Values are expressed as % change from initial values.

‡ Copper concentration of 70 to 110 μg/l.

§ Prepared from carbonyl iron powder (96% iron), Grade RX, Antara Products, General Aniline and Film Corp., N.Y.City.

liver and in serum of the animals in the "normal-copper" group remained relatively constant.

In the "low-copper" group, the serum copper decreased rapidly in the first 5 days to 48% of the initial value. During the next 35 days there was a more gradual decrease to a level of about 23 μg %. Thereafter, concentration remained at this low level. Concentration of copper in the liver decreased gradually over the first 20 days and thereafter remained constant at about 60% of the initial value.

In the animals fed a "high-copper" diet there was a progressive increase in concentration of copper in both the serum and the liver. Over the first 20 days there was a more prompt increase in serum copper than in liver copper. Thereafter, there was a slightly greater rate of accumulation of copper in the liver as compared with the serum (Fig. 1).

Anemia of significant degree was not present in the animals in the "low-copper" group in spite of an 80% reduction in serum copper and a 40% reduction in liver copper. The appearance of hypoferremia in the "low-copper" animals is in agreement with observations in copper-deficient pigs(2). Likewise, the failure of copper-depleted rats to develop hypoproteinemia is in agreement with observations in swine(2).

Discussion. Under conditions of these experiments, dietary intake of copper greatly influenced concentration of copper in the serum and in the liver. Within certain limits concentration of copper in the serum paralleled concentration in the liver. In general, a change in the intake of copper was reflected more promptly in the serum than in the liver. With a "high-copper" diet there was a progressive increase in the concentration in both the serum and the liver but after about 30 days there was a greater rate of accumulation of copper in the liver than in the serum. Thus, the capacity of the serum copper to increase beyond a certain point seemed more limited than was the capacity of the liver.

It is interesting that with a diet low in copper, concentration of copper in the liver decreased to about 60% of the initial value in 20 days but thereafter there was no further decrease. This suggests that there is a "labile" fraction of liver copper and a more "stable" fraction. In severely anemic copper-deficient pigs, the liver copper averages 5% of the normal values(2). Thus, the more stable fraction of liver copper is depleted in severely deficient animals with anemia. It should be pointed out that the experiments in swine were performed on rapidly growing, 5 day old pigs, whereas the rats used in the present experiments were not fed the low-copper diet until after the age when they grow most rapidly. This probably accounts for the lesser degree of depletion of liver copper in the rats than in the pigs.

It should be emphasized that concentration of copper in the serum and in the liver does not reflect dietary intake of copper under all conditions. Likewise, under certain circumstances there is no relationship between concentration of copper in the serum and in the liver. For example, in Wilson's disease(9), the dietary intake of copper is normal, concentration of copper in the serum is greatly reduced, and concentration of copper in the liver is greatly increased. In this disease there is a specific inability to synthesize ceruloplasmin, the copper protein of plasma. In conditions in which protein synthesis, rather than availability of copper, limits synthesis of ceruloplasmin(1), it may be expected that there will be disparity between concentration of copper in the serum and in the liver. Likewise, in conditions such as pregnancy and infection in which the synthesis of ceruloplasmin is accelerated(10), there may be disparity between dietary intake of copper and serum copper concentration and between concentration of copper in serum and in the liver.

Summary. 1. Dietary restriction of copper in adult rats was followed by prompt decrease in serum copper and a somewhat slower and less extensive depletion of liver copper. 2. Feeding of diets high in copper was followed by increase in concentration of copper in both serum and liver. The increase occurred more rapidly in serum than in liver during the first 20 days. Thereafter, rate of increase was greater in liver than in serum. 3. It is concluded that concentration of copper in serum and liver is markedly influenced by dietary

intake of copper and that there is a close correlation between serum and liver copper concentrations.

The authors are indebted to Mrs. Marilyn Dahm, Mr. George Trappett and Mr. Dale Chlarson for technical assistance.

1. Zipursky, A., Dempsey, H., Markowitz, H., Cartwright, G. E., Wintrobe, M. M., *A.M.A. J. Dis. Child.*, in press.

2. Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E., Wintrobe, M. M., *Blood,* 1952, v7, 1053.

3. Gubler, C. J., Lahey, M. E., Cartwright, G. E., Wintrobe, M. M., *Am. J. Physiol.,* 1952, v171, 652.

4. Gubler, C. J., Lahey, M. E., Ashenbrucker, H., Cartwright, G. E., Wintrobe, M. M., *J. Biol. Chem.,* 1952, v196, 209.

5. Hamilton, L. D., Gubler, C. J., Cartwright, G. E., Wintrobe, M. M., PROC. SOC. EXP. BIOL. AND MED., 1950, v65, 75.

6. Gubler, C. J., Cartwright, G. E., Wintrobe, M. M., *J. Biol. Chem.,* 1957, v224, 533.

7. Weichselbaum, T. E., *Am. J. Clin. Path., Tech. Sec.,* 1946, v16, 40.

8. Crosby, W. H., Munn, J. I., Furth, F. W., *U. S. Armed Forces Med. J.,* 1954, v5, 693.

9. Cartwright, G. E., Hodges, R. E., Gubler, C. J., Mahoney, J. P., Daum, K., Wintrobe, M. M., Bean, W. B., *J. Clin. Invest.,* 1954, v33, 1487.

10. Markowitz, H., Gubler, C. J., Mahoney, J. P., Cartwright, G. E., Wintrobe, M. M., *ibid.,* 1955, v34, 1498.

Received April 28, 1958. P.S.E.B.M., 1958, v98.

Bile Acid Metabolism, Dietary Fats, and Plasma Cholesterol Levels.* (24094)

SANFORD O. BYERS AND MEYER FRIEDMAN
Harold Brunn Institute, Mount Zion Hospital, San Francisco, Calif.

The discovery of plasma cholesterol lowering properties of ingested highly unsaturated fats(1,2) has stimulated extensive study concerning the possible mechanism by which this effect is mediated. Most research has been focused upon possible changes in plasma transport or tissue disposition of cholesterol, while little attention has been directed toward possible intra-intestinal effects of ingested unsaturated fats. However, recently Gordon *et al.*(3) have reported an increased excretion of bile salts in feces of individuals ingesting sunflower seed oil (iodine No. 135) as against those ingesting hydrogenated coconut oil (iodine No. 6). Recently, we observed(4) that when absorption of bile acids (but not of cholesterol) was diminished in rats by ileal reimplantation of the common bile duct, a marked reduction in plasma cholesterol concentration occurred. In the present study the effects of ingested highly unsaturated fats upon both the flow and constituents of bile and upon absorption of cholesterol itself are reported.

Methods. A. *Acute effect of ingested highly unsaturated fats upon intestinal absorption of cholesterol.* To determine the possible effect of a highly unsaturated fat upon intestinal absorption of cholesterol, a group of 9 healthy male adult rats (Long-Evans strain) were starved for 24 hours, then given 50 mg of cholesterol dissolved in 3 ml of soy bean oil (iodine No. 121; Liebermann-Burchard positive material = 4.6 mg/ml calculated as cholesterol). Another group of 5 rats was given the same amount of cholesterol in 3 ml of corn oil (iodine No. 120; L-B positive material = 11 mg/ml). For control purposes, one group of 5 rats was given 50 mg of cholesterol in 3 ml of warmed lard (iodine No. 59; L-B positive material = 1.2 mg/ml), and another group of 5 rats, the same amount of cholesterol in 3 ml of coconut oil (iodine No. 6; L-B positive material = 1.1 mg/ml). Immediately after feeding, all rats were subjected to cannulation of intestinal lymph duct(5) and lymph collected for 24 hours was analyzed for cholesterol(6). B.

* Aided by grants from Life Insurance Medical Research Fund and Amer. Heart Assn.