acts to dilute these constituents. The above data lend further support to the hypothesis that the % water in the fat free solids of adipose tissue is constant.

The recent observation(3) that an increase in density of adipose tissue occurs as the result of loss of fat during caloric restriction in humans could be interpreted by the above data.

The decrease in % water in fat free solids of cold treated animals reflects the contraction of extracellular fluid volume as the result of cold exposure(4).

Summary. Peri-renal adipose tissue may contain varying amounts of water, neutral fats and solids. The absolute amount of these constituents is a function of weight of tissue. Cold exposure in addition to depleting adipose tissue of neutral fat also decreases water content of fat free solids. Water and solids vary directly with each other and both vary inversely with amount of neutral fat present. It appears that water present in adipose tissue is located in tissue solids and is not associated directly with neutral fats; and that when neutral fats are added to adipose tissue they are added, with limits of this experiment, independently of water.

1. Keys, A., Brozek, J., *Physiol. Rev.*, 1953, v33, 245.

2. Pace, N., Rathborn, E. N., *J. Biol. Chem.*, 1945, v158, 685.

3. Entenman, C., Goldwater, W. H., Ayers, N. S., Behnke, Jr., A. R., *J. Appl. Physiol.*, 1958, v13, 129.

4. Bass, D. E., Herschel, A., *Physiol. Rev.*, 1956, v36, 128.

Received July 7, 1959. P.S.E.B.M., 1960, v103.

Effect of Dietary Protein Quality on Alteration of Serum Proteins and Lipoproteins in the Rat.* (25534)

E. S. ERWIN† (Introduced by A. R. Kemmerer)
Dept. of Animal Science, University of Arizona, Tucson

The physiological importance of serum proteins and lipoproteins is not fully understood. Besides providing for maintenance of osmotic pressure(1) in serum, they provide transportation mechanisms for many water insoluble nutrients such as Vit. A(2), cholesterol(3) and steroid hormones(4) to sites of tissue utilization. While many infectious diseases have been shown to alter markedly the protein fractions of sera, comparatively little attention has been given to nutritional influences. In this study protein from several single sources was fed to rats to determine effects on serum protein and lipoproteins.

Method. Four groups of rats, 4 male and 6 female, 48 days old, were fed purified rations that contained casein, lactalbumin, gluten and zein respectively as primary protein source (Table I). After 38 days the rats were sacrificed and livers and kidneys removed and immediately weighed. Serum proteins and lipoproteins were electrophoretically separated on paper strips in a Durrum cell in sodium veronal buffer (pH = 8.6; u = .076) at 2.5 ma. for 16 hours. Serum proteins were dyed with bromphenol blue while lipoproteins were dyed with oil red O for 24 hours at 37°C. The per cent of total serum and lipoprotein of each fraction was determined by scanning with an analytrol (Beckman Instrument Co.)

TABLE I. Composition of Experimental Diets.

Constituents	Ration No. 1	2	3	4
	g			
Casein	180			
Lactalbumin		192.1		
Gluten			188.9	
Zein				190.1
Mineral mix	40	40	40	40
Brewers yeast	80	80	80	80
Fiber	50	50	50	50
Sucrose	600	584.8	594.9	575.6
Fat	50.0	53.1	46.2	64.3

* Arizona Agric. Exp. Station Technical Paper No. 545.

† Present address: Monsanto Chemical Co., St. Louis, Mo.

TABLE II. Effect of Sex and Dietary Protein Quality on Liver, Kidney, Serum Proteins and Lipoproteins of Rats.

	Dietary protein				Sex	
	Casein	Lactalbumin	Gluten	Zein	♂	♀
Wt change, g						
Initial	102.0	101.7	99.4	100.2	104.6	98.5
Final	162.3	175.5	168.6	104.3 †	177.6	135.9
Kidney wt, g	.627	.649	.684	.741†	.655	.688
Liver wt, g	3.86	3.76	3.63	3.31	3.50	3.59
Protein, %	60.2	64.1	58.0	55.8	57.4	60.9
Dry matter, %	34.5	31.2	39.2	36.2	37.2	34.6
Serum proteins, %						
Albumin	64.2	65.9	67.6	61.5 †	66.7 *	63.6
Alpha globulin	7.8	7.4	8.3	9.3	8.5 *	7.9
Beta "	16.3	13.1	15.5	16.8 †	14.8	15.8
Gamma "	11.7	13.6	8.6 †	12.4	10.0	12.7 *
Lipoproteins, total	72.6 †	50.6	60.7 †	33.8	55.3	51.7
Fraction III, %	25.3	21.2	11.2 †	6.1 †	13.8	20.1 *
II, %	63.8	73.5 †	74.9 †	55.0	72.9 *	61.4
I, %	10.9 †	5.3 †	13.9	38.9	13.3	18.5

* $P < .05$. † Significantly different from rats fed other source of protein ($P < .05$).

All data were subjected to analyses of variance and Duncan's Multiple Range Test for significance(5).

Results. Sex did not influence kidney or liver weight or liver protein and dry matter composition. Almost all serum protein and lipoprotein fractions were significantly influenced by sex (Table II).

Although serum protein fractions did not vary greatly in magnitude, zein-fed rats exhibited a significantly lower serum albumin than rats fed protein from other sources. Zein-fed rats were not significantly different in beta globulin % from rats fed casein. Beta globulin % of rats fed zein was significantly lower than in rats fed gluten. Gluten-fed rats exhibited significantly lower gamma globulin than rats fed protein from other sources.

Total lipoprotein concentration of rat sera was estimated from total analytrol counts, indicative of total dye concentration. Sera from casein-fed rats possessed the greatest lipoprotein concentration (Table I), but this concentration did not differ statistically from that of gluten-fed rats. Lipoprotein content of zein-fed rat sera was significantly lower than that of sera of rats fed protein from any other source. Characteristic lipoprotein fractions of rats fed protein from various sources are presented in Fig. 1. When based on % of total lipoprotein the slowest moving lipoprotein fraction (III) was similar for rats fed casein and lactalbumin. However, these 2 dietary proteins resulted in significantly higher fraction III than either gluten or zein.

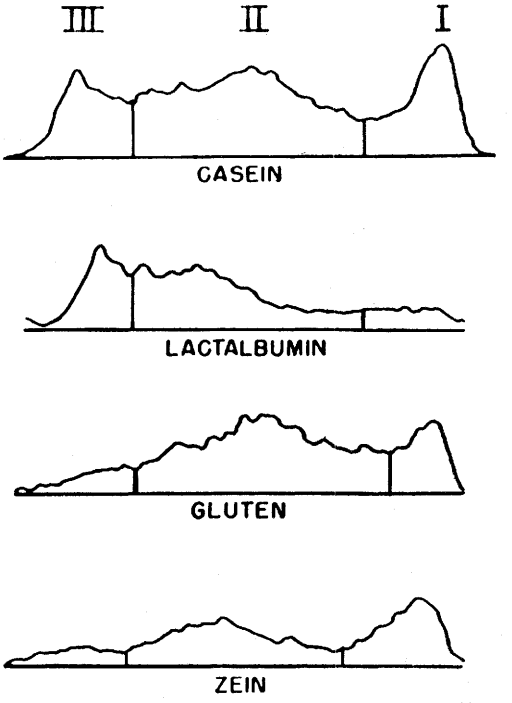

FIG. 1. Characteristic analytrol tracings of lipoprotein fractions from sera of rats fed various types of pure proteins. Fraction I was the fastest moving fraction and similar in mobility to albumin.

Sera of rats fed zein possessed significantly lower concentrations than sera from rats in any other group. Fraction II of rats fed lactalbumin and gluten was significantly higher than that of rats fed casein or zein. A significantly smaller percentage of fraction II was found in sera of zein-fed rats than in the sera of any other group.

Discussion. The importance of amount and proportion of serum proteins and lipoproteins in sera of animals is only partially understood. Diets were formulated similar to those fed by James and El Gindi(6) who reported that protein quality markedly influenced carotene utilization in the rat. Since Erwin, *et al.*(3), and others have found that albumin binds Vit. A and carotene and probably forms a transportation mechanism for these nutrients, it appeared likely that the effect of dietary protein quality was mediated through alteration in albumin content of the sera. However, the effect of dietary protein quality on alteration of albumin proportion in total serum protein could not account for differences observed in carotene utilization by the rat. Unfortunately, total serum albumin per cent was not measured.

Several investigators(7) observed that quantity of dietary protein altered serum cholesterol content. More recently, others(8) found that quality of dietary protein also influenced the level of serum cholesterol. Serum cholesterol has been found to be largely associated with certain lipoprotein fractions and, in rats, with alpha lipoprotein. The results of this study suggest that the influence of dietary protein quality on serum cholesterol is mediated through alteration of quantity and type of serum lipoproteins. That is, dietary protein affects the transportation mechanism for cholesterol and probably does not contribute to its synthesis.

Summary. Serum proteins and lipoproteins were fractionated in sera from male and female rats fed 4 pure protein sources respectively. Almost all lipo- and serum protein fractions were affected by sex. Serum protein fractions did not vary greatly in magnitude but statistically significant changes were attributed to dietary proteins. Type of dietary protein markedly altered the fractions of lipoproteins in the rats.

1. Autio, L., Korkainen, V. J., Wartiovaara, V., *Ann. Med. et Biol. Fenniae,* 1957, v35, 409 (Chem. Abst., v52, 17351).

2. Greenbaum, B. W., Geary, J. R., Grande, J., Anderson, J. T., Glick, D., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 613.

3. Erwin, E. S., Varnell, T. R., Page, H. M., *ibid.,* 1959, v100, 373.

4. Takikawa, H. Yakugaku Zasshi, 1958, v78, 1269 (Chem. Abst., v53, 6313).

5. Li, J. C., *Introduction to Statistical Inference,* 1957, Edward Bros., Ann Arbor, Mich.

6. James, W. H., El Gindi, I. M., *J. Nutr.,* 1953, v51, 97.

7. Yakota, K., Oha, S., *Kagaku,* 1958, v5, 18 (Chem. Abstr. v52, 20472).

8. Nath, N., Harper, A. E., Elvehjem, C. A., *Arch. Biochem. Biophysic,* 1958, v77, 234.

Received September 21, 1959. P.S.E.B.M., 1960, v103.

Effect of Carbon Tetrachloride on Release of Free Fatty Acids by Rat Adipose Tissue.* (25535)

MICHAEL C. SCHOTZ AND RICHARD O. RECKNAGEL†
(Introduced by George Sayers)
Research Division, Cleveland Clinic and Dept. of Physiology, Western Reserve University School of Medicine, Cleveland

Calvert and Brody(1) presented evidence that the mechanism of carbon tetrachloride hepatotoxicity involves a primary action on the central nervous system. According to their hypothesis carbon tetrachloride administra-

* Supported by grants from Nat. Heart Inst. and from Nat. Inst. of Arthritis and Metab. Dis., N.I.H., P.H.S.

† Senior Research Fellow of Nat. Inst. Health, P.H.S.