Serum Proteins and their Conjugates in Mice

Some attention has been devoted in recent years to ultracentrifugal and electrophoretic fractionation of the serum lipoproteins in various species of animals. The mouse has, however, been neglected; this seems regrettable, as the genetic differentiation of stocks and strains and the study of hereditary diseases has been developed to a greater degree in mice than in other mammalians. A preliminary comparison was therefore undertaken of the blood protein fractions, the stainable lipid fraction and the protein-bound carbohydrate in two stocks of mice; modification of this pattern by 'simple' obesity and by a complex hereditary syndrome was also studied. The simple 'regulatory' obesity is that due to the hypothalamic lesions caused by the administration of a dose of 1 mg/g body weight of goldthioglucose[1,2]. The metabolic obesity is the recessively inherited obese hyperglycemic syndrome, a condition characterized by adiposity, hyperglycemia, hypercholesterolemia, sensitivity to growth hormone, and resistance to insulin, hyperplasia of the islets of Langerhans with increased pancreatic and circulating insulin and a variety of other endocrine, enzymatic, and behavioral idiosyncrasies[1,3].

The procedures used were as follows:

Paper electrophoresis was carried out by the horizontal open strip method reported by MOINAT and TULLER[4]. The paper strips (s and S 2043 Agl) were stained for the serum protein moieties with Amido Black 10B and the excess dye removed with a phenol-acetic acid wash as reported by GRASSMANN and HANNIG[5]. Stainable lipides (lipide moieties attached to protein) were stained with Sudan Black B by the procedure described by MOINAT, APPEL, and TULLER[6], while protein-bound carbohydrates were stained according to a periodic acid-Schiff method similar to that of KOIW[7]. Areas corresponding to the uptake of the various dyes were determined with the aid of a Spinco Analytrol (Model 301-RA) using a blue filter for measuring the uptake, or absorbance, of the Amido Black 10B and of the Sudan Black B. A red filter (620 mμ) was found to be best for protein-bound carbohydrate measurements. Both areas and relative percentages were determined.

Blood samples were obtained at about three and were pooled from 6 animals for each analysis. The serum was separated and the electrophoretic resolutions carried out that same night. Data were also obtained on the effects of aging of some samples of sera and on ultracentrifugal separations. These results will be reported elsewhere[8] as portions of a larger methodological study. The sizes of sample, applied to the paper were 0·005 ml for proteins and 0·04 ml for the stainable lipides and the protein-bound carbohydrates.

Data on serum protein fractions in mice are given in Table I. Typical values for the rat and man are given for comparative purposes. It is readily seen that mice have a serum protein pattern intermediary between that of the rat and that of man. An unusually sharp β-globulin band is observed in all groups of mice. The sharpness and denseness of this band are comparable to that seen in the rat although the relative mobilities may differ. In mice, the general appearance of the β-globulin band as well as that of the α_2-globulin was first taken as an indication that both might be further resolved into two bands each. However, changing conditions such as duration of electrophoresis, voltage, and ionic strength of buffer did not produce better resolution into subfractions. Although the β-globulin fraction appeared similar to those seen in the rat in appearance and quantity, the α_1- and α_2-globulin components were more comparable to the human: the α_2-globulin component was heavier than the α_1-component. The albumin and the γ-globulin fractions are smaller in total proportion than in man.

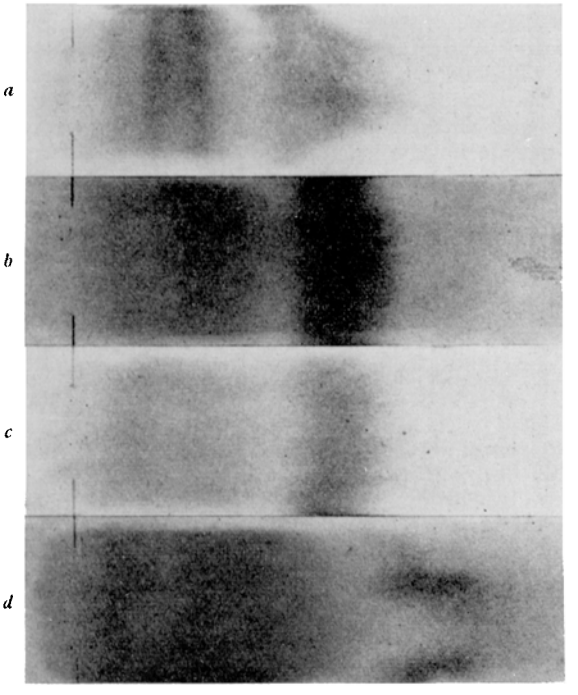
Table I

Relative percentages of serum protein fractions in 4 groups of mice

Types of Animals	Albumin	Globulins			
		α 1	α 2	β	γ
Thin Littermates . . .	40	8	14	23	15
Obese Hyperglycemic .	46	8	15	21	10
Thin Swiss	46	7	14	19	15
Goldthioglucose-obese Swiss	41	10	16	22	10
Rats	49	11	9	21	10
Man	58	5	8	12	17

Components expressed as percentages of total planimeter areas.

Table I also shows that γ-globulin values are lower in both the obese-hyperglycemic mice and the goldthioglucose hypothalamic mice than in their respective lean littermates.



Blood lipoprotein patterns in 4 groups of mice
Reading from top to bottom:
a Thin littermates of *b*;
b Obese hyperglycemic mice;
c Thin swiss;
d Goldthioglucose obese swiss.

[1] J. MAYER, Z. Vitaminforsch., in press.
[2] N. B. MARSHALL, R. J. BARRNETT, and J. MAYER, Proc. Soc. exp. Biol. Med. *90*, 240 (1955).
[3] J. MAYER, Nutr. Abstr. Rev. *25*, 597, 871 (1955).
[4] P. G. MOINAT, and E. F. TULLER, Analyt. Chem. *29*, 1655 (1957).
[5] W. GRASSMANN and K. HANNIG, Naturwiss. *21*, 496 (1950).
[6] P. G. MOINAT, W. APPEL, and E. F. TULLER, Clin. Chem., in press.
[7] E. KOIW and A. GRÖNWALL, Scand. J. clin. Lab. Invest. *4*, 244 (1952).
[8] E. F. TULLER, to be published.

Table II
Total blood lipids, blood cholesterol and total area lipoprotein in 4 groups of mice

Types of Animales	Total Lipids (mg/100 ml)	Blood Cholesterol (mg/100 ml)	Total Area Lipoprotein (cm^2)
Thin Littermates .	632 ± 148	116 ± 10	130
Obese Hyperglycemic	955 ± 239	170 ± 24	240
Thin Swiss	872 ± 71	112 ± 41	145
Goldthioglucose Swiss	1214 ± 55	125 ± 24	340

Duplicate strips were stained with Sudan Black B to locate the lipides. Table II gives data on total lipids, stainable lipides and cholesterol in the blood of these different groups. Methods and data on total lipids and cholesterol have been published[9,10]. The distribution of the lipides (Figure) is similar to that of the rat: lipide materials are found throughout the complete serum protein pattern. The picture is thus in contrast to the sharp α- and β-peaks seen in strips from human sera. There are again differences between groups of mice. The strips from the serum of the thin littermates of obese-hyperglycemic mice showed little lipide in the albumin region but 3 rather distinct bands in the globulin area; 2 in the β-region and 1 in the α-2. The strips from the swiss mice showed heavy lipid staining in the albumin region with light bands in the β- and α 2-globulin regions. Strips from both types of obese mice showed much heavier lipide staining than did strips from thin mice. The albumin fraction was particularly heavy in the obese hyperglycemic mice, somewhat lighter and more mobile in the goldthioglucose obese mice. No distinct bands were observed in the β-or α-region for either type obese mice.

In summary, electrophoresis reveals well-defined patterns of blood protein and blood lipids in mice; these are in many ways intermediary in appearance between patterns obtained for rats and patterns obtained for man. There are strain differences and also differences between obese mice and non-obese mice. The latter appear to be non-specific for the type of obese syndrome, differences due to the increase in fat transport in obese animals overshadow specific differences such as the hypercholesterolemia of obese-hyperglycemic mice contrasted to the normal cholesterol of goldthioglucose mice[1].

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Résumé

L'électrophorèse sur papier permet un fractionnement des protéines et des lipides du sang chez les souris. La distribution des diverses fractions est intermédiaire entre celle du rat et celle de l'homme. Il existe des différences entre diverses souches de souris, ainsi qu'entre souris obèses et non obèses. Les méthodes employées ici, quoique faisant ressortir les anomalises accompagnant l'obésité, ne permettent pas de différencier entre les divers types d'obésités.

[9] J. MAYER and D. J. SILIDES, Exper. *14*, 96 (1958).
[10] J. MAYER, C. ZOMZELY, and F. J. STARE, Exper. *13*, 1 (1957).

The Photoelectric Theory of Photosynthesis IV. The Chromophore Area of Chlorophyll

The Photoelectric Theory of Photosynthesis. It has been postulated by the author in the photoelectric (or photoconductive) theory[1] of photosynthesis, that light-activated chlorophyll transfers electrons to an appropriate oxidant and removes electrons from water. It was pointed out that the primary process of photosynthesis may thus be viewed as a flow of electrons activated by light, with the chlorophyll functioning as a sort of conducting bridge between two half cells, in one of which a water molecule is oxidized by loss of two electrons ($H_2O \rightarrow 2\,e^- + 2H^+ + 1/2\,O_2$), and in the other an oxidant[2], intimately associated with the chlorophyll in the chloroplast, is reduced by gaining two electrons. Stated in an alternative way, a stream of photons strikes the solid chlorophyll phase and creates a stream of conduction electrons which reduce the oxidant. The electron holes left in the chlorophyll phase are constantly eradicated by electrons flowing from water to the chlorophyll[3].

This theory has proved useful in explaining certain experimental results, such as, for example, the recently observed quenching of chlorophyll fluorescence by triphenyl tetrazolium chloride in the presence of hydrazine[4]. Here the organic chloride acts as the electron acceptor and hydrazine replaces water as electron donor. The photoconductive theory has recently been placed on a much stronger experimental basis by measurements on the electron spin resonance of photoactivated chlorophyll by COMMONER, HEISE, and TOWNSEND[5].

Electronic Spectrum of Mg in Chlorophyll. It has been demonstrated by the author[6] that there is an extremely close correspondence of the chlorophyll absorption bands with the emission lines of electronically excited states of the Mg^0 atom and the Mg^+ ion in the visible and near ultraviolet regions of the spectrum. Tables I and II show these comparisons.

These spectroscopic considerations provide excellent evidence that the actual site of initial electron loss by the chlorophyll molecule is at the *Mg atom.* Such electron loss would result in the intermediate conversion of the chlorophyll molecule to a positively charged radical-ion representing an oxidized chlorophyll species:

$$\mathrm{Chl-Mg^{\circ}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{+}} + e^- . \qquad (1)$$

In addition to this *direct photoelectric process*, the following equations representing the photosynthetic mechanism were proposed (Ox represents the electron acceptor):

$$\mathrm{Chl-Mg^{\circ}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{*}} \qquad (2)$$

$$\mathrm{Chl-Mg^{*} + Ox} \longrightarrow \mathrm{Chl-Mg^{+} + Ox^{-}} \qquad (3)$$

$$\mathrm{Chl-Mg^{*} + Ox^{-}} \longrightarrow \mathrm{Chl-Mg^{+} + Ox^{=}} \qquad (4)$$

$$\mathrm{2\,Chl-Mg^{+} + H_2O} \rightarrow \mathrm{2\,Chl-Mg^{\circ} + 2\,H^{+} + 1/2\,O_2} \qquad (5)$$

$$\mathrm{Chl-Mg^{+}} \xrightarrow{h\nu} \mathrm{Chl-Mg^{+*}} \qquad (6)$$

$$\mathrm{Chl-Mg^{+*} + Ox} \longrightarrow \mathrm{Chl-Mg^{++} + Ox^{-}} \qquad (7)$$

$$\mathrm{Chl\text{–}Mg^{++} + H_2O} \rightarrow \mathrm{Chl\text{–}Mg^{\circ} + 2\,H^{+} + 1/2\,O_2} \qquad (8)$$

[1] L. S. LEVITT, Science *118*, 696 (1953).
[2] Possibly the oxidant is 6,8-thioctic acid, the prosthetic group of pyruvic oxidase.
[3] L. S. LEVITT, Abstracts of Papers, Minneapolis Meeting of the Amer. chem. Soc., p. 67C (1955).
[4] E. FUJIMORI, J. Amer. chem. Soc. *77*, 6495 (1955).
[5] B. COMMONER, J. J. HEISE, and J. TOWNSEND, Proc. nat. Acad. Sci., Wash. *42*, 710 (1956).
[6] L. S. LEVITT, Science *120*, 33 (1954).