creasing the copper supply does the same, particularly at the lower level of iron. The uptake of iron is greatly reduced as the time of incubation is shortened.

Walsh *et al.* (*1*) reported a correlation between reticulocyte count and iron uptake when using blood from pernicious anemia patients in remission, and concluded that their results could be interpreted in physiologic terms. In our studies with rabbit blood this correlation was not obtained. The iron uptake by blood cells was considerable and was increased by the inclusion of copper in the culture medium. As the amount was influenced by several other factors and was far in excess of that which could possibly be used in hemoglobin synthesis, this result is not interpreted in physiologic terms. Our results indicate that the mechanism which determines iron uptake must be fairly complex.

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Manuscript received September 2, 1953.

Radioactive Gold in Filter Paper Electrophoresis Patterns of Plasma

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When radioactive colloidal gold is injected into the pleural or peritoneal cavities of patients with effusions, small but measurable amounts of radioactivity circulate in the blood. Andrews *et al.* (*1*) have indicated that less than 1% of the given dose is in the blood at one time. This finding was confirmed in a series of patients treated at the Mount Sinai Hospital (*2*). Less than 0.2% of the given dose in the serous cavity was encountered in the circulating blood.

The gold in the blood is in the plasma; the red blood cells and white blood cells contain no measurable radioactivity. When trichloracetic acid is added to plasma the gold is in the protein precipitate. To determine whether gold in plasma is coprecipitated with or bound to protein presents a problem. If the gold is bound to protein it is of further interest to know which protein fractions bind the gold.

Attempts to solve these problems by using chemical methods of protein separation have yielded inaccurate results. Separating the proteins by electrophoresis would be a cumbersome procedure. Filter paper electrophoresis, however, is a method that simplifies the separation of plasma proteins (*3*) and lends itself to the determination of which protein fractions bind radioactive gold (*4*). In this method a current is passed through a strip of filter paper which has been soaked in buffer solution. The ends of the filter paper dip into positive and negative electrode vessels. Samples of protein-containing solution are applied to the paper, and proteins migrate with the current. Separation of the protein fractions is satisfactory in a few hours. These fractions can be dyed differentially for identification.

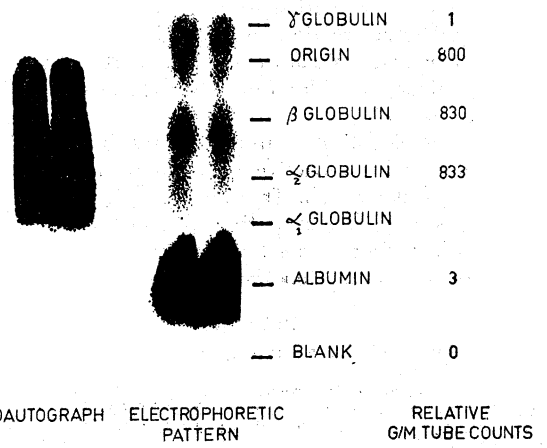

FIG. 1.

Radioactive colloidal gold (Au^{198}) was added to normal plasma *in vitro* (500 μc Au^{198} to 200 cc plasma), and samples of this solution were precipitated with trichloracetic acid at time intervals following the mixture of these two components. Counting the radioactivity of these precipitated samples showed that the uptake of radioactive gold in the proteins was maximal within 15 min. The proteins in the original solution containing gold (before precipitation) were then separated by applying about .030 cc of the plasma to filter paper. Figure 1 shows the filter paper electrophoretic pattern of these separated proteins and identifies the fractions. The distribution of radioactive gold in this pattern was determined by Geiger-Muller tube counts of various areas in the electrophoretic pattern as indicated in Fig. 1. The thin window (5 mg/cm^2) of the tube was 6 mm in diameter. A radioautograph of the same electrophoretic pattern confirmed the binding of radioactive colloidal gold to the α and β globulin fractions of the plasma proteins; no gold was bound to the γ globulin or albumin. As indicated in Fig. 1, considerable radioactivity remained at the site of the originally applied droplet of plasma on the filter paper.

This process was repeated with plasma from a patient who had received an intrapleural injection of 60 mc of Au^{198} 24 hr previously. The distribution of Au^{198} in the plasma protein in this patient is similar to the distribution in the plasma to which gold had been added *in vitro*.

In the filter paper electrophoretic pattern of ascitic fluid from a patient who had carcinoma of the pancreas, the ascitic fluid was aspirated from the peritoneal cavity one day after the intraperitoneal injection of 142 mc of Au^{198}. The gold is distributed in essentially the same fractions as in the blood. Some of the gold is unfixed to the globulin and remains at the point of application (origin) of the ascitic fluid on the filter paper.

[1] The author acknowledges the cooperation of Arthur W. Ludwig in this work.

A similar distribution of radioactive gold in the α and β globulin fractions was obtained in a specimen of fluid aspirated from the pleural cavity of a patient 6 days after the intrapleural injection of 56 mc of Au^{198}.

The method of filter paper electrophoresis has established that radioactive colloidal gold is bound to the α and β globulin fractions of plasma proteins. Even more important, this previously introduced method is a simple tool for studying the distribution of radioactivity in blood and its proteins.

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Manuscript received October 16, 1953.

The Multiple Etiology of Obesity: Production of Two Types of Obesity in Littermate Mice[1]

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A number of types of experimental obesity have been studied in this and other laboratories (*1*): hypothalamic obesity in the rat, yellow obesity (dominant) in the mouse, the hereditary (Mendelian recessive) obese-hyperglycemic syndrome,[2] thalamic obesity in the monkey, hereditary (Mendelian recessive) obesity in the Shetland sheep dog, immobilization obesity in the rat, and goldthioglucose obesity in the mouse. Unfortunately, these obesities have usually been produced in different animal species or at least in different strains, and their metabolic similarities and differences have rarely been studied simultaneously. We wish to report here, first a comparison between two types of obesity in different strains of mice, and next the establishment of these two types in littermate animals with direct comparison of metabolic characteristics.

The hereditary obese hyperglycemic syndrome is characterized in particular by extreme overweight (limit weights of 50–115 g as compared to 25–30 g) and by a form of hyperglycemia sensitive to diet and to growth hormone and extremely resistant to large doses of insulin (*2*). Recent evidence (*3*) indicates that these animals are characterized by oversecretion of a hormone secreted by the alpha cells of the islets of Langerhans, of which growth hormone is the tropic hormone.

Goldthioglucose obesity (*4*) is produced by injection of the LD_{50} dose of goldthioglucose. About 30% of the animals that survive injection become obese, the limiting weight is in the 50–95 g range. No metabolic study has been conducted in these animals.

The blood glucose levels, effect of insulin, and effect of growth hormone were determined in 3 groups of Swiss mice: goldthioglucose-treated obese animals (range 40–54 g), goldthioglucose-treated nonobese animals (range 20–24 g), and untreated controls (range 20–28 g). These animals had been injected 8 mo previously with 15 mg of goldthioglucose.[3] The same procedure was conducted simultaneously in 3 groups of animals of the genetically obese stock, obese (80–115 and 40–55 g), and nonobese (18–27 g) mice representing age and weight controls, and described in Table 1, and 1 group of nonobese littermates of young hereditarily obese animals, made obese (40–56 g) by goldthioglucose injection. This last group represented the obese survivors of 5 equal groups of 20 animals injected with 15, 25, 35, 40, and 50 g of goldthioglucose. None of the animals injected with 15 or 25 mg had developed obesity; all animals injected with 50 mg died. Eight of the 20 animals injected with 35 mg and 9 of the 20 animals injected with 40 mg died. Three animals injected with 35 mg and 9 animals

TABLE 1. Effect of growth hormone on blood glucose in goldthioglucose obesity and in the hereditary obese hyperglycemic syndrome.

Animals			Blood glucose (mg %)	
Type	Number	Weight (g)	Untreated	After growth hormone
Goldthioglucose treated, obese Swiss	12	45.3 ± 4	118 ± 9	110 ± 14
Goldthioglucose treated, nonobese Swiss	12	22.8 ± 0.7	107 ± 11	105 ± 6
Untreated Swiss	12	24.0 ± 2	105 ± 7	97 ± 6
Hereditary obese-hyperglycemic, 3½ mo old (weight controls)	8	45.2 ± 3	160 ± 20	303 ± 4
Hereditary obese-hyperglycemic 9 mo old (age controls)	8	92.0 ± 11	298 ± 41	485 ± 82
Nonobese *ob ob* littermates	12	23.8 ± 4	115 ± 7	116 ± 16
Nonobese *ob ob* littermates made obese by goldthioglucose	8	45.2 ± 5	124 ± 15	106 ± 12

[1] Supported in part by grants-in-aid from the National Institute of Arthritis and Metabolic Diseases and the National Heart Institute, National Institutes of Health, U.S. Public Health Service, Bethesda, Md., and the Nutrition Foundation, Inc., New York City.

[2] The term hyperglycemia, which represents a condition either found naturally in the obese animals or immediately elicited by small doses of growth hormone to which the nonobese animals are insensitive (*5*) is much to be prefered to the term "diabetes" previously used. If by diabetes is meant the insulin-free condition, the obese animals are not diabetic.

[3] Supplied by Schering Corp., Bloomfield, N. J. These animals were given by G. Brecher, National Institute of Arthritis and Metabolic Diseases, Bethesda, Md.