From the two photographs one sees at once the striking loss of absorption in ultraviolet light typical of all the cells in the x-radiated preparations examined from 48 hr through 120 hr. This loss of absorption is present in both the cytoplasm and the nucleus, and the clearly defined structures, which absorb so beautifully in the normal cell, are virtually completely absent in the second figure. Together with this change, one gains the impression here—as in the other photographs—that the nuclei of the irradiated cells are swollen. No attempt was made to define in more precise terms this impression, as sufficient data and technical methods are not available to establish this point.

It should be pointed out here that if one photographs dying control material a picture similar to Fig. 2 is obtained—indicating that at these wavelengths the ultraviolet absorption picture of the process of cell death is the same in radiation cell death and cell death due to other factors.

These photographs are reproduced in black and white; when color translated, the black areas appear green, with some gentle shading of yellow and red in the cytoplasm. Clearly visible in color are the cell borders, the cytoplasm, the nuclear membranes, nucleoli, and chromatin. As shown by Caspersson, intense absorption of ultraviolet light at these wavelengths is characteristic of nucleoproteins, both RNA and DNA, and in fact represents in the main absorption by the purine and pyrimidine components of the nucleic acids (*4*).

From these data we are able to say that the ultraviolet light absorbing cell structures in these preparations do not change from normal as determined by the Polaroid color translating ultraviolet microscope until 48 hr after 60,000 r of x-radiation. From 48 hr through 120 hr they undergo a profound change.

This profound change—quantitatively and qualitatively the same from 48 hr on—is reflected in the striking contrast between the photographs in Figs. 1 and 2. The almost complete loss of all absorption in both the cytoplasm and the nucleus, together with the impression of nuclear swelling, is indeed striking. Its interpretation is difficult. Three possible explanations may be offered. We shall discuss them in the order which we consider the most likely.

1) This change may represent an absolute loss of both RNA and DNA amounting to almost complete absence. Nucleoproteins are known to be affected by x-radiation both *in vivo* and *in vitro*, and in dying cells loss of nucleoproteins seems inevitable (*5, 6, 7*).

2) This change might conceivably represent an alteration in the physical state of the nucleoproteins with reference to the more liquid portions of the cell, thus producing an ultraviolet absorption effect simulating loss of nucleoproteins.

3) The change may be accounted for by assuming that the purine and pyrimidine rings—in reality the absorbing structures—have been so changed by the x-radiation that they no longer give their characteristic absorption.

In order to define better the underlying mechanisms of this nucleoprotein change, similarly prepared control and irradiated cultures were studied histochemically at the same postirradiation intervals. No postirradiation change was observed in the acid phosphatase by Glick's (*8*) modification of Gomori's method, in the nonspecific esterase by the method of Barrnett and Seligman (*9*), in the lipid by Sudan Black B staining, or in the protein bound sulfhydryl by the method of Barrnett and Seligman (*10*).

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The Immunochemical Heterogeneity of Human Plasma β-Lipoprotein[1]

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From the protein fractions obtained by the partition of human plasma in ethanol-water mixtures at low temperatures (*1–3*), solutions of β-lipoprotein were isolated and purified in the preparative ultracentrifuge (*4, 5*). Gofman *et al.* (*6*) have reported that human serum lipoproteins show considerable heterogeneity with respect to flotation rates in the ultracentrifuge in a sodium chloride solution of density 1.06. Oncley *et al.* (*7*), on centrifuging relatively concentrated β-lipoprotein solutions for about 18 hr in a glycine-sodium chloride medium of density 1.3, separated three β-lipoproteins of different flotation rates. In an attempt to gain greater understanding of the relationships between lipoprotein metabolism and certain aspects of normal and pathologic human physiology, several investigations have been conducted in this laboratory on the metabolism of human β-lipoprotein. This report presents evidence that the plasma β-lipoprotein fraction is composed of a number of lipoproteins differing markedly in their immunochemical characteristics.

Rabbit antiserum vs human plasma β-lipoprotein

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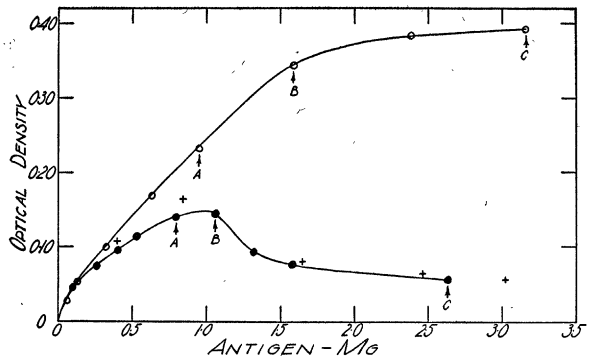

FIG. 1. Precipitation curves obtained by reacting rabbit antiserum vs human plasma β-lipoprotein with preparations of β-lipoprotein obtained from two different pools of human plasma. Arrows: supernatants of these reaction mixtures tested as in Table 1. ○: preparation BS0; ●: preparation BS1.

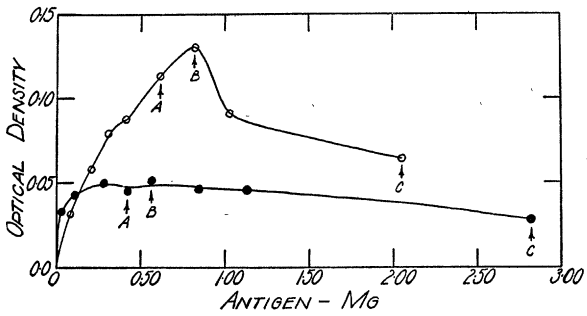

FIG. 2. Precipitation curves obtained by reacting rabbit antiserum vs human plasma β-lipoprotein with preparation BS3 (○) and the lipid-poor fraction, preparation G3 (●), separated during the purification of BS3. Arrows: supernatants of these reaction mixtures tested as in Table 1.

was obtained by using a freshly purified preparation of β-lipoprotein, BSX, separated from pooled plasma by the method of Oncley *et al.* (*5*). The lipoprotein was dissolved in 0.15 *M* NaCl, and this solution was given to rabbits subcutaneously at 10-day intervals, 25 mg/kilo body weight/injection for 4 injections. The first injection was administered within a week of final purification of the lipoprotein in the ultracentrifuge; the solution was kept at 0° to 2° C at all times between injections. Rabbit antisera vs human serum γ-globulin, preparation L413-II-1, and crystallized human serum albumin, preparation Decanol 10 (*8*), were obtained by employing aluminum hydroxide-adsorbed antigens (*9*); rabbits were injected subcutaneously at 7–10-day intervals, 25 mg antigen/kilo body weight/injection for 5 injections.

After the rabbit antisera had been prepared, purified β-lipoprotein was obtained from several different pools of human plasma (*5*) and the preparations kept separately. They were designated BS1, BS3, and BS0. In one instance the lipid-poor greenish layer, always found at the bottom of the preparative cell after the ultracentrifugal purification of β-lipoprotein, was removed and designated G3 (*4, 5*).

The spectrophotometric method (*10*) for analysis of the specific precipitates was used. Antigen-antibody reaction mixtures, 1.0 ml of an appropriate dilution of antigen added to 1.0 ml of antiserum, were incubated at 37° for 1 hr and then placed at 1° C for 18 hr. Specific precipitates were washed twice with cold saline, being centrifuged for 30 min at 4000 rpm at 0° C to recover the precipitates.

The precipitation curves obtained by reacting pooled rabbit antiserum vs human plasma β-lipoprotein with preparations BS1, BS3, and BS0 are illustrated in Figs. 1 and 2. The curve obtained in the reaction between this antiserum and the lipid-poor fraction, preparation G3, separated during the purification of BS3, is shown in Fig. 2. Since β-lipoprotein has been shown to undergo profound changes on standing (*5*), it should be noted that these preparations were kept at 1° C for various periods of time before first being tested: BS3 and G3 for 1 week, BS1 for 2 weeks, and BS0 for 8 months. That the differences between the precipitation curves of BS1 and BS0 were not due simply to aging and oxidation of the lipoproteins of BS0 is indicated in Fig. 1: BS1 was kept at 1° C for an additional 6 weeks and then retested with the same antiserum. The differences in the BS1 curves on aging would seem hardly enough to account for all the differences between BS1 and BS0. The Tyndall effect of BS1 after 6 weeks had become very intense, indicating that changes had in fact taken place without approaching the immunological characteristics of BS0. It will also be noted that the precipitation curves of BS3 and G3 differed from each other as well as from those obtained with BS1 and BS0.

The supernatants of those reaction mixtures indicated by arrow in Figs. 1 and 2 were tested with the β-lipoprotein preparations by conventional ring tests, and the results are shown in Table 1. The marked heterogeneity of the β-lipoproteins became quite evi-

TABLE 1

THE IMMUNOCHEMICAL HETEROGENEITY OF HUMAN SERUM β-LIPOPROTEIN AS INDICATED BY RING TESTS FOR REACTANTS IN EXCESS

Supernatant tested with	Precipitation curve: antiserum + BS1			Precipitation curve: antiserum + BS0		
	Reaction mixture			Reaction mixture		
	A	B	C	A	B	C
Antiserum	+	+	+	−	+	+
BS1	−	−	−	+	±	−
BS3	+	+	+	−	−	−
BS0	+	+	+	+	−	−
G3	+	+	+	+	−	−
	Precipitation curve: antiserum + BS3			Precipitation curve: antiserum + G3		
Antiserum	+	+	+	+	+	+
BS1	+	+	+	+	+	+
BS3	±	−	−	+	+	+
BS0	+	+	+	+	+	+
G3	+	±	±	±	±	−

dent, and the data substantiated the evidence obtained from the differences in their precipitation curves.

The multiplicity of antigens was also indicated by layering various concentrations of the β-lipoprotein preparations over antiserum in agar according to the Oudin procedure as described by Munoz and Becker (*11*). By this technique 5 bands were readily revealed in BS0 and 6 bands in BS1.

No γ-globulin could be detected in BS1 with rabbit antiserum vs human serum γ-globulin; BS1 contained about 0.2% human serum albumin as determined immunochemically (*10*). The rabbit antiserum vs β-lipoprotein revealed no demonstrable antibodies vs human serum albumin, human serum γ-globulin, or crystallized human serum β-metal-combining globulin (*12*). There was some cross reaction between rabbit anti-β-lipoprotein and the α-lipoprotein fraction, IV-1-1 (*1*), but, owing to the demonstrated heterogeneity of this fraction electrophoretically, this reaction could not be interpreted satisfactorily.

It has been found in this study, therefore, that the β-lipoprotein fraction of normal human plasma represents a class of β-lipoproteins differing in their immunochemical characteristics. Since several preparations of β-lipoprotein obtained from different pools of plasma were studied, the data suggest that the immunochemically reactive components were present in varying ratios in the individual plasmas. The data also indicate that normal human plasma contains a lipid-poor protein that is immunochemically related to the protein moiety of β-lipoprotein; the evidence is insufficient to state this relationship any more positively.

It has been suggested that definite distributions of physical heterogeneity of circulating lipoproteins as demonstrated by the ultracentrifuge is intimately associated with certain pathological states such as atherosclerosis (*6*). The relationships between immunochemical heterogeneity and physical heterogeneity await further investigation, but analysis of the data presented here suggests that immunochemical heterogeneity exists within the various flotation "classes."

The correlation between circulating lipoprotein and human disease is extremely interesting in view of the observation that β-lipoprotein is a component of the cell nuclei of almost all human tissues (*13*). Since the level of individual plasma proteins is a reflection of tissue metabolism, the finding of an altered pattern or level of a particular protein may be only a secondary factor in a disease state rather than a primary or etiologic factor. It is also true, however, that the altered level of the plasma protein, although secondary to tissue metabolism, can itself give rise to characteristic disease as evidenced in hemophilia, afibrinogenemia (*14*), and agammaglobulinemia (*15*). The possible relationships between tissue metabolism and hyperlipoproteinemia in disease await further study.

Kunkel (*16*) fractionated human serum with zephiran and ultracentrifugation and obtained a β-lipoprotein which proved antigenic in rabbits; the rabbit antiserum was then used to estimate lipoproteins in normal and pathological sera. For the estimation of an antigen immunochemically, it must be ascertained that the antigen is either immunochemically homogeneous or that the ratio of its reactive components remains constant. Although the β-lipoprotein employed as an antigen in this study was prepared differently from that of Kunkel, the basic immunochemical criteria for use of antiserum in quantitative estimations were not fulfilled for the β-lipoproteins. By proper absorption techniques, however, it may be entirely feasible to render such antisera specific.

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The Effect of Indoleacetic Acid and Amount of Solar Radiation on Heterosis in the Snapdragon (*Antirrhinum majus* L.)[1]

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In the course of studies with hybrid snapdragons *Antirrhinum majus* L., it became apparent that light was a factor in determining the degree of heterosis and the degree of reaction of the plants to indoleacetic acid. Heterosis is the difference between the mid-parent mean and F_1.

Since auxins are believed to be essential to cell elongation and since one of these substances, indoleacetic acid, is inactivated by riboflavin in the presence of light (*1*), it is possible that a difference in auxin level may account for the phenomenon of heterosis, and that these phenomena may be influenced by the amount of light received by the plant.

In order to test this general hypothesis, two inbred lines of *Antirrhinum majus* L. and their hybrid were chosen as the experimental test subjects. Three experiments were conducted under different light conditions to obtain data on height and dry weight of the parents and of the F_1.

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