trinsic accuracy of the data presented, a further caution should be strongly emphasized. The uncertainties involved in inferring radiation dose from distance alone are too large to support conclusions beyond the previously reported qualitative one that those survivors who received large doses of radiation—that is, who were within 1500 meters of the hypocenter—had a significantly higher incidence of leukemia than those beyond that distance, who received relatively little or none (*3*). The relationship of incidence to distance as presented in Table 1 cannot be given a more quantitative interpretation because there are too many variables, as yet unresolved, which cannot be ignored.

For example, the presently available estimates of the air dose in Hiroshima have a large uncertainty, the magnitude of which is itself not yet definite. Also, experimental dosimetry studies at Oak Ridge National Laboratory emphasize the need for detailed information, such as is being collected by the Atomic Bomb Casualty Commission, concerning the shielding situation of any particular survivor at any distance. It is conceivable that the radiation received within a light frame house (the most common shielding situation) may vary from an amount almost equaling the outside air dose to one equal to the outside air dose attenuated by perhaps a factor of two, depending on the position of the person in the house.

In determining the relationship of radiation exposure to the incidence of leukemia, such detailed data must be examined not only for each leukemic survivor but also for enough of the population at risk to permit calculation of statistically significant incidence rates. Until this information becomes available from the dosimetry program, it is premature to attempt precise quantitation of dose-effect relationships in radiation leukemogenesis on the basis of the Hiroshima and Nagasaki radiation-exposed populations (*8*).

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Stimulating Action of Soluble Antigen-Antibody Complexes on Normal Guinea-Pig Smooth Muscle

During the course of a study on the disappearance of soluble antigen-antibody complexes in rabbits, it was observed that normal rabbits exhibited moderate-to-severe shock following the intravenous injection of solutions containing soluble antigen-antibody complexes prepared by dissolving precipitates in excess antigen. The significance of these observations was not fully appreciated until Germuth and McKinnon (*1*) reported their work on the production of shock in guinea pigs following the injection of soluble antigen-antibody complexes formed in excess antigen.

In view of these findings, the investigation described in this report (*2*) was made to determine the effect of such soluble complexes on isolated strips of smooth muscle from the intestine of normal guinea pigs. The Schultz-Dale technique of in vitro tissue anaphylaxis provides a more sensitive measure of the irritability of soluble antigen-antibody complexes than other techniques, and it was thought that this technique might indicate whether nontoxic soluble complexes could be obtained that would permit continuation of the in vivo studies on the disappearance and fate of soluble complexes in rabbits.

The antigen used in this study was recrystallized bovine serum albumin (BSA) (*3*). A pool of antiserum was obtained from rabbits following 12 intravenous injections (of 10 mg each) given over a period of about 4 weeks. Quantitative precipitin curves were obtained by the method described by Lanni and Campbell (*4*). Each tube contained 3 ml of the antigen dilution and 3 ml of undiluted antiserum. The diluent used was saline-borate buffer (pH 8.4; μ = 0.006 borate and μ = 0.16 NaCl). After the tubes had been kept 48 hours at 4°C, they were centrifuged for 45 minutes at 3000 rev/min (1600 *g*) in a refrigerated centrifuge, and the clear supernates were decanted into a separate set of tubes. The precipitates were washed and then analyzed for total nitrogen by Nesslerization (*4*). The guinea pigs used for this work were normal nonsensitized animals, each weighing between 400–500 g. Each animal was killed by a blow on the head, and 30 to 40 cm of the lower portion of the small intestine was carefully removed and kept moistened at all times with warm modified Tyrode solution (*5*). The intestinal contents were carefully removed by flushing the intestine with three or four 25-ml portions of fresh Tyrode solution.

Each supernate was tested, by the method of Campbell and McCasland (*6*), on fresh duplicate strips of guinea-pig ileum (3 to 4 cm in length) from two different animals. The strips were immersed in a 120-ml muscle bath containing Tyrode solution at 37°C, and a stream of O_2 (90 percent) plus CO_2 (10 percent) was used to aerate and stir the bath. The experimental procedure was to allow the muscle strips to equilibrate in the bath for 15 minutes, add 2 ml of the supernate to be tested, and record the subsequent contraction of the muscle strip for a 10-minute period. Five micrograms of histamine was then added to the bath as a means of obtaining a value for maximal contraction. The stimulating effect of soluble complex was expressed as a percentage of the maximal contraction obtained with histamine.

Control tests were made with solutions containing only antiserum or normal rabbit serums, varying in amounts from 0.08 to 3.64 times the amount of protein in

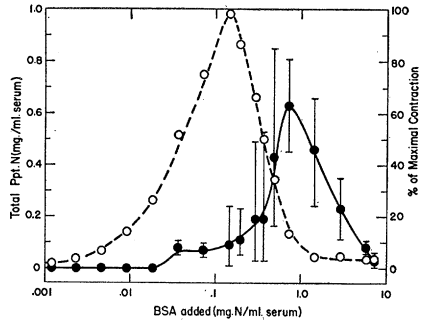

Fig. 1. Relationship between the precipitin curve (open circles) and smooth-muscle contraction (closed circles). The contraction of the guinea-pig ileum is expressed as a percentage of the maximal response obtained with histamine and is the average for six intestinal strips. The range is indicated by the vertical lines.

Table 1. Effect of BSA–anti-BSA supernates on smooth muscle of guinea pig.

Antigen N added per milliliter of serum (mg)	Total ppt of N (mg per ml of serum)	Tests on supernates		
		Excess antigen or antibody	Schultz-Dale (% of max.)*	Skin reaction
7.3465	0.037	Antigen	3	++
5.8772	0.037	Antigen	8	+
2.9386	0.048	Antigen	23	++
1.4693	0.048	Antigen	46	+++
0.7347	0.135	Antigen	63	+++
0.4898	0.346	Antigen	43	+++
0.3673	0.500	Antigen	19	+++
0.2939	0.663	Antigen	19	+
0.1959	0.867	Antigen	11	±
0.1469	0.982	Antigen	9	±
0.0735	0.750	Antibody	7	+
0.0368	0.515	Antibody	8	++
0.0184	0.263	Antibody	0	+
0.0092	0.140	Antibody	0	+
0.0046	0.067	Antibody	0	+
0.0023	0.037	Antibody	0	+
0.0012	0.020	Antibody	0	+
Antiserum control	0.00	Antibody	0	−
Histamine (1.0 μg)			100	++++
1% NaCl				−

* Based on the reaction to 5 μg of histamine as 100 percent. The values shown are the average for six strips of ileum.

the supernatant solutions. Control tests were also made on bovine serum albumin solutions alone and on mixtures of bovine serum albumin and normal serum. In no instance did these control systems cause any smooth-muscle contraction. The supernates were also tested for their skin reactivity by intradermal injection into the skin of normal guinea pigs which had previously received an intravenous injection of Evans blue (Table 1).

The data (Fig. 1) showed that the principal activity of the supernates occurred in the range of slight-to-moderate antigen excess. The stimulating action rapidly decreased to zero in antibody excess and nearly so in extreme antigen excess. It was noted that there was a time lag of from 30 to 90 seconds between the addition of the test solution and the contraction of the muscle. This lag was much greater than that ordinarily seen with histamine or when antigen is added to sensitized tissue, and this suggests that a different mechanism may be involved. All tissues which were allowed to react to an initial exposure of supernate, and which were then washed and exposed again, failed to contract; this is suggestive of an immune reaction. This of course is similar to typical desensitization of muscle from a sensitized animal in that the muscle becomes refractory to antigen after the primary exposure. Further studies must be made before the real significance of soluble antigen-antibody complexes in hypersensitivity reactions is understood. For example, the problem arises whether soluble complexes in excess antibody (that is, toxin-antitoxin systems) would give reactions, and whether the failure to obtain reactions in cases of extreme antigen excess is due to an inhibitory effect of free antigen or to the nature of the complexes (see Singer and Campbell, 7). The same reaction can be obtained with soluble complexes formed by dissolving carefully washed precipitates in excess antigen. However, no study has been made of solubilized precipitates formed in a decomplemented serum.

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N-Terminal Amino Acid and Lipid Composition of Lipoproteins from Chyle and Plasma

One of the major theories of fat transport is that lipoproteins rich in triglycerides (S_f 20–400 and chylomicrons) are transformed into higher density lipoproteins by the successive removal of triglycerides (*1*). Two primary problems arising from this hypothesis are the relationships between two of the major classes of lipoproteins found in human plasma [*D*, 1.006–1.063 (S_f 0–20) and *D*, 1.063–1.21 (HDL)] to each other and to the lipoproteins of density less than 1.006 which appear to be the principal vehicles for triglyceride transport. If any classes of lipoproteins are interconvertible then they must contain identical protein moieties and differ only by reason of their lipid constituents. The amino acid end group analyses of Avigan *et al.* (*2*) and Shore (*3*) and studies utilizing labeled lipoproteins have demonstrated conclusively that the predominant components of two classes, *D*, 1.019–1.063 and *D*, 1.063–1.21 are not interconvertible. These results exclude the possibility that all of the lipoproteins of human plasma represent a continuous spectrum containing a common protein. However, the relationship of these lipoproteins to those having densities less than 1.006 (chylomicrons and S_f 20–400) has not been reported.

This paper contains the results of N-terminal amino acid analyses of the protein moieties of the complete spectrum of lipoproteins from the chylomicrons of chyle to the highest density lipoproteins (*D*, 1.063–1.21) in plasma.

Chylomicrons from chyle were obtained from a patient with a chylothorax associated with Hodgkins disease. Fractions I, II, and III (Fig. 1) were obtained from three separate patients with idiopathic hyperlipemia. Fractions IV, V, VI, and VII were obtained from the plasma of blood bank donors at the National Institutes of Health.

Chylomicrons from chyle and plasma (fraction I) were isolated by layering the chyle or plasma under saline at density 1.006 and centrifuging at 50,000 *g* for 30 minutes in the Spinco 21 rotor. The packed chylomicrons were then suspended in 30 percent sucrose and layered under 10 volumes of 0.055*M* phosphate, *p*H 7.0, and recentrifuged at 50,000 *g* for 30 minutes. This process was repeated up to eight times until a constant protein concentration was obtained. After removal of the chylomicrons, the plasma was centrifuged at density 1.006 for 24 hours at 80,000 to 90,000 *g*. The packed yellow material (fraction II) at the top of the tube was suspended in 30 percent sucrose and layered under 0.055*M* phos-