tramuscular or intraperitoneal injections of autologous skin and adjuvants.

Summary. 1) Eighty-nine guinea pigs were injected intracutaneously, intramuscularly or intraperitoneally with autologous skin in emulsion with adjuvants. Thirty-eight guinea pigs were injected intracutaneously or intramuscularly with control emulsion, not containing skin. Subsequently an autograft was done on each animal. No significant differences were noted in acceptance of autografts between animals injected with skin emulsion and control emulsion. 2) Irritancy tests using scraping and burning of the skin did not produce different results in the test animals and the control animals. 3) Under the conditions of these tests no autosensitization to skin could be demonstrated in guinea pigs.

1. Whitfield, A., *Brit. J. Dermat.*, 1922, v34, 331.

2. Haxthausen, H., *Acta dermat.-venereol.*, 1955, v35, 271.

3. Templeton, H., Lunsford, C., Allington, H., *A.M.A. Arch. Dermat. and Syph.*, 1949, v59, 68.

4. Cormia, F. E., Esplin, B. M., *ibid.*, 1950, v61, 931.

5. Hecht, R., Sulzberger, M. B., Weil, H., *J. Exp. Med.*, 1943, v78, 59.

6. Voisin, G., Maurer, P., *La Semaine des hopitaux*, 1955, no. 33, 1909.

7. Cavelti, P. A., Cavelti, E. S., *Arch. Path.*, 1945, v39, 148.

8. Burkey, E. L., *J. Allergy*, 1934, v5, 466.

9. Freund, J., Lipton, M. M., *J. Immunol.*, 1955, v75, 454.

10. Thomas, L., Paterson, P. Y., Smithwick, B., *J. Exp. Med.*, 1950, v92, 133.

11. Lumsden, C. E., Kabat, E. A., Wolf, A., Bezer, A. E., *ibid.*, 1950, v92, 253.

12. Waksman, B. H., Morrison, L. R., *J. Immunol.*, 1951, v66, 421.

13. Fink, M. A., Smith, P., Rathlauf, M. V., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 590.

14. Freund, J., Lipton, M. M., Thompson, G. E., *J. Exp. Med.*, 1953, v93, 711.

15. Billingham, R. E., Medawar, P. B., *J. Exp. Biol.*, 1951, v28, 385.

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Chromatographic Differences Between Radioiodinated Albumin Preparations and Normal Human Serum Albumin. (23716)

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Radioiodinated human serum albumin has been extensively used to investigate albumin metabolism in the normal and in diseased states(1-3). Albumin preparations used in these studies have been indistinguishable from normal human serum albumin in many respects but not in all(1,2,4,5). The recent introduction of anion-exchange adsorbents(6) which have proved very useful in the chromatographic separation and characterization of serum proteins(7,8), made possible further evaluation of radioiodinated human serum albumin preparations. In the present study, utilizing the anion-exchange adsorbent, diethylaminoethyl-cellulose(6), the chromatographic distribution of radioiodinated human serum albumin was investigated and found to differ markedly from the distribution of native serum albumin.

Materials and methods. The radioiodine-labeled human serum albumin preparation used in these studies has been commercially prepared by the following procedures. Albumin was separated at the Cutter Laboratories from pooled plasma by a modification of the cold ethanol technic of Cohn(9). Following addition of stabilizers (acetyltryptophan and sodium caprylate) the albumin was heated to 60°C for 10 hours to minimize the possibility of transmission of homologous serum hepatitis(10). Iodination of the albumin was performed at the Abbott Laboratories(11) and included exposure of the albumin to potassium iodide and sodium hypochlorite solutions and passage over an ion-exchange column. This preparation, referred

to as RISA-T_3, contained 1 atom of iodide for every 5.5 molecules of albumin. Half a ml of this iodinated albumin solution, containing 25 μc and 25 mg of albumin, was added to 25 ml of fresh normal human serum containing 950 mg of albumin. Anion-exchange (DEAE-) cellulose chromatography was carried out as described in detail elsewhere(8). The solution containing I^{131}-labeled albumin and serum was dialyzed for 21 hours at 4°C with several changes of the 0.01 M pH 8 sodium phosphate buffer, and 20 ml of the dialyzed preparation was added to a column containing 16 g of DEAE-cellulose adsorbent.* Gradient elution with a flow rate of 20-25 ml/hr was employed with one liter of 0.01 M pH 8 sodium phosphate buffer in the mixing chamber and 500 ml of 0.30 M monosodium phosphate in the reservoir. Effluent fractions of 8 ml were collected and examined for protein content by measurement of the optical density at 280 mμ in a Beckman DU spectrophotometer. Electrophoretic identification(8) of the chromatogram fractions was performed after ultrafiltration. Total protein determinations were made by standard biuret procedure(8). *Radioactivity* of 2 ml aliquots of the chromatographic fractions was measured in a thallium-activated sodium iodide well scintillation counter. Localization of the radioactivity in the whole serum and chromatographic fractions was ascertained after paper electrophoresis by means of an end window Geiger-Muller counter, employing a continuously recording counting-rate meter, and the distribution of radioactivity was compared with protein distribution, as determined by bromphenol blue staining. Radioactivity was determined on duplicate unstained and stained strips without detection of significant difference.

Results. The chromatographic distribution of the normal serum proteins, of serum albumin and of radioiodinated albumin are illustrated in Fig. 1. The pattern of the serum chromatogram is typical of that obtained with normal serum when this technic is employed.

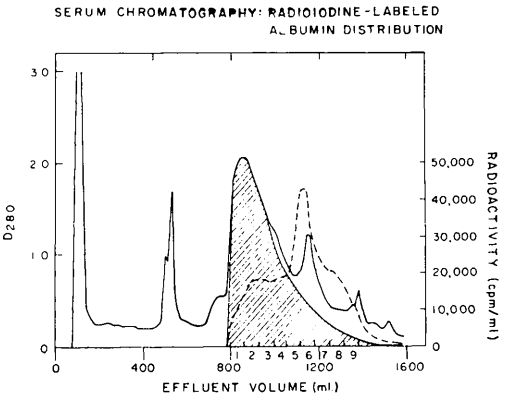

FIG. 1. Chromatogram of normal human serum and I^{131} albumin. Protein distribution is indicated by solid line, albumin distribution by shaded area, and radioactivity (I^{131} albumin) by broken line. The radioactive albumin represents only a very small fraction (less than 1%) of the total amount of albumin in the serum. The serum proteins distribution pattern is normal(8). The near coincidence of the major peak of radioactivity elution with a peak of serum protein elution is probably fortuitous for this serum protein peak is normally present and contains largely alpha-1, alpha-2 and beta globulins.

and the distribution of native serum albumin also agrees with previous experience(8). However, the difference between serum albumin and radioiodinated albumin distribution is readily apparent.

The effluent region containing almost all of the radioactivity (800 to 1400 ml) was subdivided into 9 equal parts. Aliquots from the effluent fractions in each part were pooled, concentrated by ultrafiltration and examined by electrophoresis. There was no demonstrable radioactivity in the ultrafiltrate. After electrophoresis of these pools the distribution of radioactivity was found to coincide with the location of albumin in every instance. The quantity of albumin and radioactivity in each of these regions are presented in Table I. Combined fractions 1-4 were composed almost entirely of albumin yet were of low specific activity. Although the greatest radioactivity (I^{131} albumin) was found in combined fractions 5 and 6, there was a further relative increase in radioactivity in pools 7-9 as the total albumin content decreased further in the final portions of the chromatogram. Fig. 2 illustrates the electrophoretic protein and radioactivity distribution in the regions where albumin elution was greatest (865-928 ml)

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TABLE I. Distribution of Albumin and Radioactivity in Combined Fractions of Chromatogram Effluent.

†	Combined fraction Effluent vol (ml)	Albumin % of protein present	Albumin Amt of albumin* (mg)	Radioactivity Total cpm (×10³)	Radioactivity cpm/mg albumin
1	801- 864	98	173	469	2.7
2	865- 928	99	191	936	4.91
3	929- 992	100	156	951	6.07
4	993-1056	98	103	1003	9.73
5	1057-1120	90	63	1543	24.49
6	1121-1184	53	41	1775	43.40
7	1185-1248	70	29	1188	40.95
8	1249-1312	74	21	964	45.90
9	1313-1376	21	7	499	71.31

* Obtained by determining total protein content (biuret) and albumin content by electrophoresis.
† Chromatogram region indicated on Fig. 1.

and where the radioactivity elution was greatest (1121-1184 ml). The total recovery of I^{131} albumin from the column was 91% of the amount applied.

Discussion. The difference observed in the present studies, between the chromatographic distribution of iodinated albumin preparations and native human serum albumin, was marked and striking. Two other iodinated albumin preparations have been similarly chromatographed and the distribution of iodinated albumin in each instance was found to resemble that reported here. Whether this change is attributable to the addition of radioiodine or to some step in the separation and sterilization of albumin remains to be determined.

Oxidizing agents such as hydrogen peroxide, used for converting iodide to iodine in I^{131} albumin manufacture, have been shown to change the biologic properties of rabbit albumin(12). It cannot be determined from the present study whether the 5-fold excess of hypochlorite is responsible for the changes observed.

Certain immunochemical and biochemical properties of human or mouse serum albumin may be altered following iodination to a level averaging 15 or more iodine atoms per albumin molecule.† However, in the RISA-T3 preparation used in this study only one iodine atom was present for each 5.5 molecules of albumin making it unlikely that excessive iodination is the reason for the differences between native and iodinated albumin shown in Fig. 1. Although on the average there is less than one iodine atom per albumin molecule it is possible that heavy iodination of some albumin molecules may have altered their chromatographic properties. It is also possible that iodination occurred primarily on albumin molecules with distinctive chemical and chromatographic properties, either normally present or produced during albumin separation and sterilization. Iodination in-

† Steinfeld, J. L., unpublished observations.

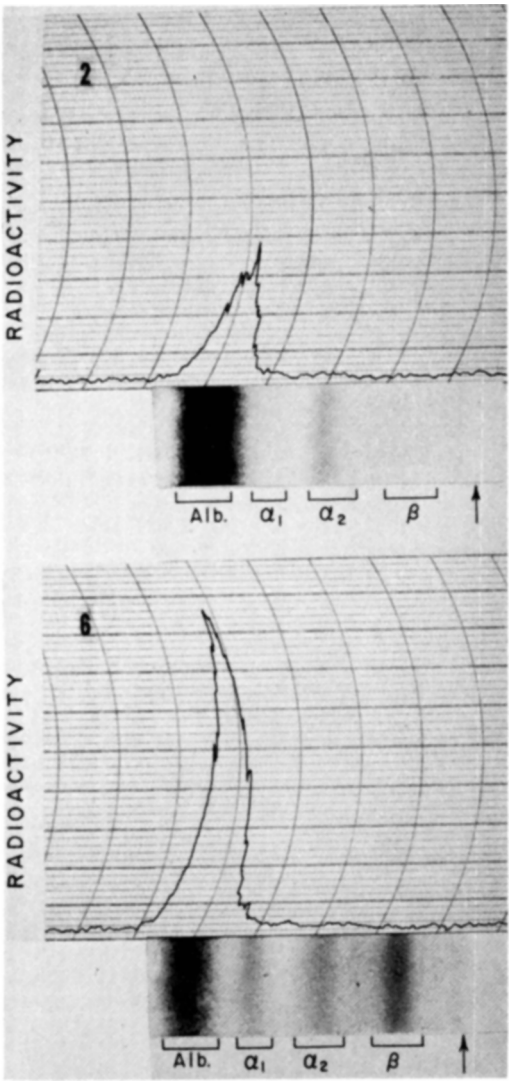

FIG. 2. Electrophoretic protein and radioactivity distribution are compared in chromatogram regions of greatest albumin content (Region 2) and of greatest radioactivity content (Region 6).

creases the dissociation of the phenolic hydroxyl groups(13). The resultant increased acidity of the iodinated molecules may be responsible for the separation from the bulk of non-iodinated molecules on the DEAE-cellulose column.

Radiation may alter albumin. A total dose of 50.000 roentgen equivalent physical (rep) to albumin in a solution of 0.2 mg albumin ml can alter its biologic properties(14). However, increasing the albumin concentration to 5 mg ml partially protects against the damaging effects of irradiation. The RISA-T3 preparation used here was exposed to a relatively low radiation dose, receiving approximately 10.000 rep in a solution containing 50 mg of albumin per ml. Also, the biologic characteristics of this I^{131} albumin preparation compare favorably with those previously reported(2). Following intravenous administration to man of RISA-T3 preparations, a constant rate of iodinated albumin degradation during 30-day periods of observation has been repeatedly observed.† This would suggest that the iodinated albumin preparation that was chromatographed had not been severely damaged by radiation, for radiation damaged albumin preparations contained variable amounts of rapidly degraded iodinated albumins.

Studies to determine which of the many possibilities discussed above were responsible for altering the iodinated albumin so that its chromatographic behavior differed from that of native serum albumin are being undertaken. Further, the significance of these chromatographic differences in terms of biologic properties remains to be elicited.

Summary. The chromatographic behavior of radioiodinated albumin preparations was found to differ markedly from that of native serum albumin on anion-exchange (DEAE-) cellulose adsorbent columns. The significance of this observation is discussed.

1. Sterling, K., *J. Clin. Invest.*, 1951, v30, 1228.
2. Berson, S. A., Yalow, R. S., Schreiber, S. S., Post, J., *ibid.*, 1953, v32, 746.
3. Steinfeld, J. L., *Proc. Am. Assn. Cancer Res.*, 1956, v2, 149.
4. Gabrieli, E. R., Goulian, D., Jr., Kinersly, T., Collet, R., *J. Clin. Invest.*, 1954, v33, 136.
5. Merchant, W. R., Masouredis, S. P., Ellenbogen, E., *ibid.*, 1957, v36, 914.
6. Peterson, E. A., Sober, H. A., *J. Am. Chem. Soc.*, 1956, v78, 751.
7. Sober, H. A., Gutter, F. J., Wyckoff, M. M., Peterson, E. A., *ibid.*, 1956, v78, 756.
8. Fahey, J. L., McCoy, P. F., Goulian, M., *J. Clin. Invest.*, Feb., 1958.
9. Cohn, E. J., Strong, L. E., Hughes, W. L., Mulford, D. J., Ashworth, J. N., Melin, M., Taylor, H. L., *J. Am. Chem. Soc.*, 1946, v68, 459.
10. Murray, R., *Bull. N. Y. Acad. Med.*, 1955, v31, 341.
11. Steinfeld, J. L., Flick, A. L., Paton, R. R., Milch, R. A., Beach, F. E., Tabern, D. L., *Ann. N. Y. Acad. Sci.*, 1957, v70.
12. McFarlane, A. S., *Biochem. J.*, 1956, v62, 135.
13. Hughes, W. L., *Ann. N. Y. Acad. Sci.*, 1957, v70, article 1.
14. Yalow, R. S., Berson, S. A., *J. Clin. Invest.*, 1956, v35, 746.

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Effect of p-Dimethylaminoazobenzene and β-Naphthylamine on Rat Liver Sulfhydryl Concentration.* (23717)

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Various experimental procedures and numerous agents will influence the total non-protein sulfhydryl (TNPSH) content of rat and mouse livers(1-8). Scalding, ligation trauma, exposure to cold or shock, restraint and subcutaneous injections of adrenalin, have been shown to cause a marked drop in the total non-protein sulfhydryl concentration. Over the past 2 years(9,10), research conducted in this department has revealed

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