STABILITY OF ^{125}I-LABELED INSULIN USED IN RADIOIMMUNOASSAY OF INSULIN

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Radioimmunoassay of insulin (*1–9*) is routinely employed for the determination of plasma insulin using high-specific-activity porcine or bovine insulin labeled with either ^{131}I or ^{125}I. Insulin labeled with radioactive iodine is prone to radiation damage resulting in the loss of its immunological activity. Damage during iodination of insulin with radioiodine and on subsequent storage of the labeled insulin has been recognized and methods for its purification have been described (*8,10–12*). During radioimmunoassay of insulin using ^{125}I-insulin, we observed on several occasions that stored radioinsulin even after the usual purification failed to react with the antibody. Since such a problem may be encountered in radioimmunoassay of insulin, our observations on this type of deterioration of radioinsulin are described below.

Iodine-125-labeled bovine insulin with specific activity of 50 μCi/μg (5 μCi/ml) was purchased from the Radiochemical Centre, Amersham, England. As soon as it was received it was stored at −30°C for not more than seven days. Once it was taken out from the deep freeze it was stored at 4°C without any dilution and after purification was used for radioimmunoassay. Insulin was purified before each assay according to the method of Banerjee (*10*). Stock ^{125}I-labeled insulin was incubated with freshly prepared iodoacetamide treated plasma and the damaged insulin bound to plasma proteins and the free insulin was separated on a sephadex G-75 column employing 0.025 *M* phosphate buffer pH 7.5 for elution. Undamaged ^{125}I-insulin was employed for the radioimmunoassay after appropriate dilution. Immunoassay was carried out essentially according to the method described by Genuth et al (*8*) employing sephadex G-75 column for separating antibody bound and free insulin. The antibody employed for the assay was guinea pig antipork insulin antiserum purchased from Burroughs Wellcome & Co., London.

When radioinsulin after incubating with iodoacetamide plasma was separated on sephadex G-75, an elution pattern with only two radioactive peaks was normally obtained (Fig. 1). The damaged insulin was eluted with a peak at 5 or 6 ml (Fraction 1), and the undamaged insulin was eluted with a peak at 11 or 12 ml (Fraction 2). All the radioactivity

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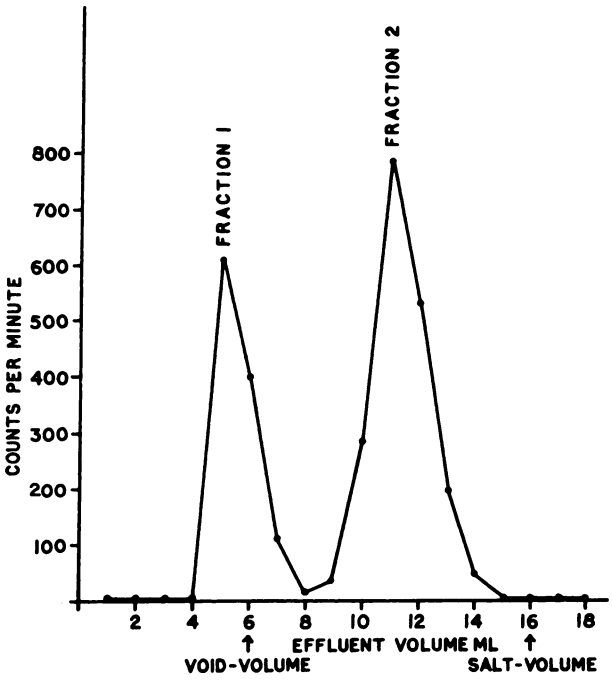

FIG. 1. Typical pattern of separation of damaged and free radioinsulin on sephadex G-75. Stock ^{125}I-insulin (0.1 ml) was incubated with 0.05 ml of iodoacetamide treated plasma at 37°C for 30 min and passed on sephadex G-75 column (1 × 12 cm). Column was eluted with 0.025 *M* phosphate buffer containing bovine serum albumin.

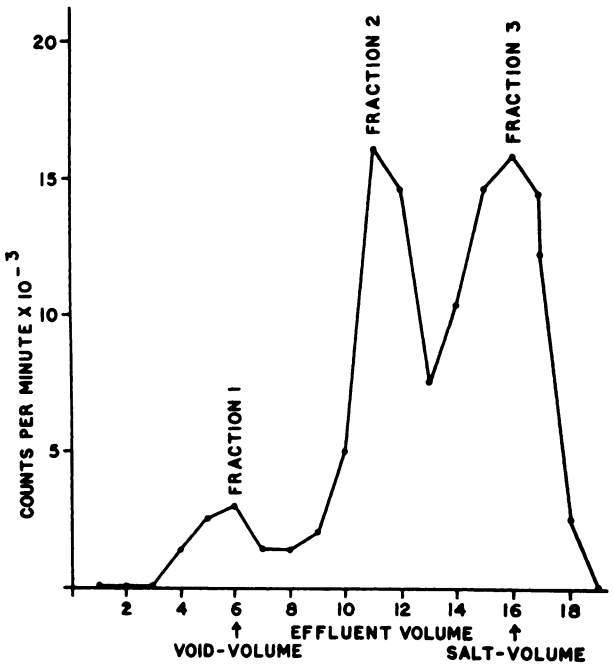

FIG. 2. Elution pattern of aged radioinsulin on sephadex G-75 column. Details same as Fig. 1 caption.

was accounted for by these two fractions. When stock ^{125}I-insulin was purified and the above pattern of separation was obtained, a satisfactory binding of radioinsulin (Fraction 2) with antibody was observed. Some batches of insulin stored at 4°C for some time, however, showed a different elution pattern with peak radioactivity appearing at 6 and 16 ml.

The pattern of separation of ^{125}I-insulin during the purification procedure was determined at periodic intervals. It was observed that ^{125}I-insulin on prolonged storage gave rise to a third radioactivity peak at 16 ml (Fraction 3) in addition to the usual two peaks observed with relatively fresh ^{125}I-insulin (Fig. 2). As shown in Table 1 the proportion of the third fraction increased with time at the expense of the second fraction.

The three fractions in stored ^{125}I-insulin preparation were separated and tested for antibody binding and electrophoretic behavior. Fraction 2 was immunologically reactive and was retained at the origin on paper electrophoresis whereas Fraction 3 was immunologically inactive and moved away from the origin. Fraction 1 on the other hand when subjected to electrophoresis was partly retained at the origin and partly moved (Fig. 3). The presence of a small amount of iodide was noted in all the three

TABLE 1. PROGRESSIVE DETERIORATION OF RADIOINSULIN WITH TIME

Days of storage	% of total radioactivity		
	1st fraction	2nd fraction	3rd fraction
	Batch 1		
0	12.0	88.0	Nil
15	18.2	81.8	Nil
30	23.0	77.0	Nil
40	28.0	72.0	Nil
45	26.0	74.0	Nil
52	12.0	Nil	88.0
60	13.0	Nil	87.0
	Batch 2		
0	2.8	97.2	Nil
15	3.8	96.2	Nil
30	6.2	60.0	33.8
40	9.4	36.0	54.6
45	9.0	30.2	60.8
50	10.2	10.4	79.4

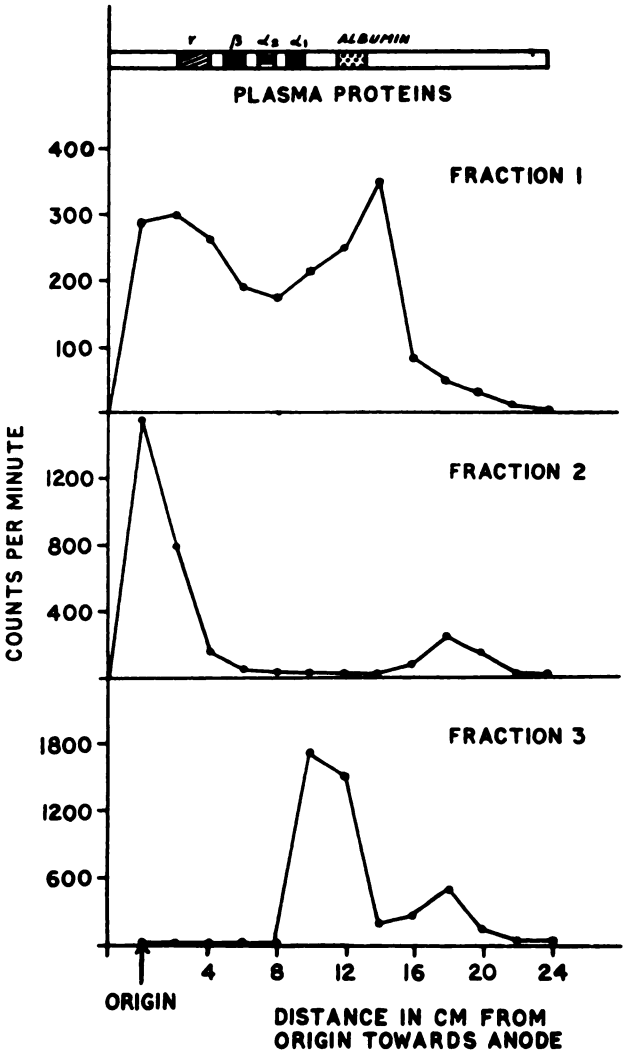

FIG. 3. Electrophoretic pattern of three fractions of radioinsulin separated on sephadex column. Each fraction was applied on filter paper and electrophoresis was carried out in Beckman electrophoresis cell for 14 hr at 150 volts using veronal buffer pH 8.6. One-centimeter strips were cut and counted in well scintillation counter.

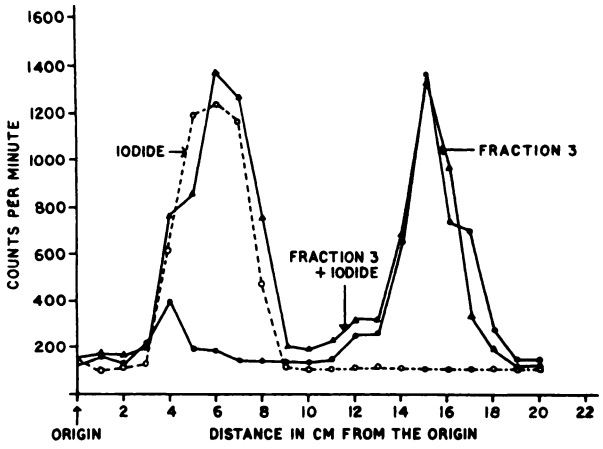

FIG. 4. Paper chromatographic separation of third fraction and iodide in butanol, acetic acid, and water (78:5:17) system for 18 hr. One-centimeter strips were cut and counted in well scintillation counter.

fractions on paper electrophoresis. The paper chromatographic separation of the third fraction with or without added radioactive iodide is shown in Fig. 4. The rf value of the third fraction was distinctly different from that of the iodide.

The type of damage resulting in the loss of immunological and plasma proteins binding property observed on prolonged storage may have resulted from self-irradiation of the insulin molecule. Since radioinsulin was stored with merthiolate, deterioration due to bacterial contamination may be excluded. The damage of the type described was not observed in all the batches of radioinsulin and also there was variation in the period by which the deterioration commenced. The Fraction 3 present in aged ^{125}I-insulin reported in this study should represent a small molecular weight degradation product of radioinsulin since it gets eluted with the salt peak on sephadex G-75 column. Its electrophoretic and chromatographic behavior strongly indicates that this fraction is not iodide.

The present observations are of practical importance for immunoassay of insulin since they indicate that poor assays obtained with some insulin preparations may be due to a type of damage that does not seem to have been recognized before. It is therefore necessary to check the radioinsulin before it is used for the immunoassay. Use of neutron activation (*13*) for the radioimmunoassay of insulin employing insulin labeled with stable ^{125}I may overcome this problem of radiation damage.

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