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Experience with a commercial preparation of ^{125}I-labelled human albumin for study of albumin metabolism

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SYNOPSIS Evaluation of a commercial preparation of ^{125}I-labelled albumin for use in the study of albumin metabolism is described. In eight subjects with normal albumin metabolism the proportion of the dose of radioiodide excreted was stable throughout a period of 17 days, indicating that there was no excessive denaturation of the iodinated albumin. Characteristics of albumin metabolism—pool sizes, catabolic rate, etc—were in agreement with currently accepted normal values. It is concluded that this preparation of iodinated albumin is suitable for metabolic use.

In most recent studies of albumin metabolism with iodinated albumin a commercial preparation of albumin (Behringwerke trocken 'reinst') has been used after iodination of the protein either by the iodine monochloride method of McFarlane (1964) or by a procedure using chloramine T as oxidizing agent (Bocci, 1964). This approach has been shown to be satisfactory (Rossing, 1967).

^{125}I- and ^{131}I-labelled albumins produced by the Radiochemical Centre (Amersham) are intended primarily for the measurement of plasma volume, and, although the manufacturer considers that the preparation and handling of the labelled albumin are such that it should be satisfactory for metabolic studies, no guarantee of suitability is given. This commercial preparation of ^{125}I-labelled albumin is currently being used by several workers for the study of albumin metabolism, although there are no available reports of its evaluation. Particularly in a small metabolic laboratory a commercial source of ^{125}I-labelled albumin would be an advantage, because patients are studied at irregular intervals and because care is needed in sterilizing the iodinated albumin for injection into man.

Subjects

Eight subjects, who could be assumed to have normal albumin metabolism, were selected. Five (three female and two male) were members of the laboratory staff and were aged 21-25 years. None showed either past or present evidence of any disease and all had normal serum concentrations of albumin. Two male patients and one female patient with both clinical and radiological evidence of osteoarthritis of the knee joints were also included. Their ages were 34, 60, and 65 years, respectively. They had no symptoms of other diseases and in particular there was no evidence of involvement of the other joints in any disease process and their serum albumin concentrations were normal. The subjects were given oral KI (30 mg twice daily) for two days before and throughout the period of the radioiodinated albumin study.

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Materials and Methods

Five batches of ^{125}I-labelled albumin were evaluated. Subjects 1-3 received aliquots from one batch and subjects 4 and 6 were injected with aliquots of a second batch. Three further batches of radioiodinated albumin were used in the studies on the three other volunteers. Details of albumin preparation and iodination are given by the manufacturer. The albumin, prepared by ether fractionation of plasma at -5-0°C, was obtained from the Lister Institute (London) and denatured material formed on pasteurization was removed by further precipitation. The specified chemical and radiochemical purity of the ^{125}I-labelled albumin is satisfactory.

Approximately 25 μCi ^{125}I-labelled albumin was injected into an antecubital vein. The contents of the syringe were weighed and after injection the residue was washed to a suitable volume with bovine

serum albumin (2 g/l, Armour) and the ^{125}iodide measured as described to determine the exact dose administered. An aliquot of ^{125}I-labelled albumin was retained to act as a standard. Ten minutes after injection a blood specimen was taken to allow determination of plasma volume. The radioactivity at 10 minutes was multiplied by 1·015 to obtain the value at zero time (Hobbs, 1967). Further 10 ml blood specimens were removed without using anticoagulants at 2 hr, 6 hr, and daily thereafter for a minimum of 17 days. Twenty-four-hour urine collections were obtained throughout this period. All specimens were kept at $-20°$ until the study was complete and the radioactivity was measured in one batch to minimize overall error and to avoid the necessity of correction for radioactive decay. The ^{125}iodide content of duplicate 2 ml samples of serum or urine was measured in a well-type, thallium-activated sodium iodide crystal used in conjunction with a pulse-height analyser, scaler-timer, automatic turntable, and printer (Ekco). Counting error was less than 1% and efficiency was 43%. Data were converted to μCi/vol, μCi/g, etc, by means of a program written for use with an electronic calculator (Wang).

The serum albumin concentration was measured by the elution technique of Webster (1965) after electrophoresis on cellulose acetate. The plasma albumin content was calculated from the mean value together with the value for plasma volume. The ratio of urine radioactivity to mid-time serum specific and total radioactivity allowed calculation of the absolute and fractional catabolic rates on each day (Campbell, Cuthbertson, Matthews, and McFarlane, 1956). The absolute catabolic rate is the mass of albumin catabolized per unit time; the fractional catabolic rate is the fraction of the plasma albumin pool catabolized per unit time. Mean values for these catabolic rates were also calculated by the method of Matthews (1957), assuming a three- or four-compartment mammillary model. This also allowed calculation of extravascular albumin content and of rates of transfer between the intravascular and extravascular 'compartments'.

Results

The mean urinary excretion of ^{125}iodide as a percentage of the dose injected was 4·66 (SEM 0·18) in the first 24 hours. An excretion of less than 5% in this period is one of the criteria of an acceptable preparation (McFarlane, 1965). The ratio of excreted to serum activity remained relatively constant throughout the 17-day period of study. Fluctuations in the ratio are considered to be due mainly to errors in urine collection, since measured urine nitrogen and creatinine contents usually showed parallel variations. When the total body activity, ie, 1 cumulative urinary excretion, was calculated and plotted versus time, the slope was the same as that of the final exponential of the plasma activity-time curve. Nevertheless, the slope of the total body curve in some subjects showed slight initial curvature, which may be due to rapid catabolism of a small proportion of denatured molecules. This, however, is apparent in many published studies (eg, Cohen, Freeman, and McFarlane, 1961).

Results for albumin content and catabolic rate calculated by the method of Matthews (1957) are given in table I. The results for catabolic rates are similar to those calculated by the method of Campbell *et al* (1956). A comparison of the results for albumin content and catabolic rate with those which are currently accepted by most workers is given in table II; where necessary published values have been recalculated so that results are in a comparable form. The values for albumin content and catabolic rate found in this study did not differ significantly from these published values.

Results for exchange rates found in this investi

Subject[1]	*Sex*	*Weight* (*kg*)	*Plasma Volume* (l)	*Plasma Concentration* (*g/l*)	*Content* (*g/kg*)		*Catabolic Rate*	
					Intravascular	*Extravascular*	*Fractional* (d^{-1})	*Absolute* ($g/kg\ d^{-1}$)
1	F	50·5	2·117	45	1·88	2·14	0·103	0·194
2	F	59·1	2·792	44	2·08	2·66	0·088	0·183
3	F	65·0	2·347	42	1·52	2·18	0·098	0·149
4	M	54·5	2·493	44	2·02	2·50	0·107	0·216
5	M	70·0	3·280	43	2·01	2·29	0·086	0·173
6	F	82·0	2·661	39	1·27	2·15	0·093	0·118
7	F	71·9	3·220	40	1·79	2·21	0·083	0·149
8	M	50·8	2·274	43	1·92	3·41	0·103	0·199
Mean		63·0	2·648	42·5	1·81	2·44	0·095	0·173
SEM		4·0	0·151	0·7	0·10	0·15	0·003	0·011

Table I *Results obtained for normal albumin metabolism*

[1]Subjects 1-5 were laboratory staff, subjects 6-8 were patients with osteoarthritis.

Study	*Content*[1] *(g/kg)*		*Catabolic Rate*[1]	
	Intravascular	*Extravascular*	*Fractional* (d^{-1})	*Absolute* ($g/kg\ d^{-1}$)
This series[2] n = 8, M + F	1·81 ± 0·10	2·44 ±0·15	0·095 ±0·003	0·173 ± 0·011
Cohen *et al* (1961) n = 11, M + F	1·89 ± 0·07	2·79 ±0·27	0·104 ± 0·004	0·185 ± 0·009
Takeda and Reeve (1963) n = 13, M	1·60 ± 0·04	2·20 ±0·11	0·089 ± 0·003	0·142 ± 0·003
Rossing (1967) 19F	1·8 ± 0·07	2·2 —	0·085 ± 0·003	0·15 ± 0·005
n = 34 15M	2·0 ± 0·08	2·8 —	0·084 ± 0·001	0·17 ± 0·008

Table II *Comparison of current results with accepted values for albumin metabolism*

[1]Mean ± SEM
[2]Results are not significantly different from published values.

gation can be compared directly with those of Cohen *et al* (1961), since the same model (Matthews, 1957) was used in each study. The values were derived assuming the existence of two extravascular pools, ie, a three-exponential plasma curve, and are given in table III. The discrepancy between the two series lies in the characteristics of the rapidly exchanging pool, which is particularly susceptible to the influence of denaturation. Both size and exchange rate from plasma are smaller in the present series.

	This Series[1]	*Cohen* et al *(1961)*[1]	*Difference*
Rapidly exchanging pool			
Ratio	0·42 ± 0·04	0·58 ± 0·04	Significant
Exchange rate (d^{-1})	0·71 ± 0·09	1·30 ± 0·09	Significant
Slowly exchanging pool			
Ratio	0·98 ± 0·12	0·86 ± 0·08	NS[2]
Exchange rate (d^{-1})	0·26 ± 0·03	0·34 ± 0·03	NS
Total extravascular pool			
Ratio	1·40 ± 0·08	1·45 ± 0·09	NS
Exchange rate (d^{-1})	0·97 ± 0·10	1·64 ± 0·10	Significant

Table III *Characteristics of extravascular albumin expressed relative to the intravascular content of albumin*

[1]Mean ± SEM
[2]Not significant

Discussion

The agreement between values established for content and catabolic rate with this commercial ^{125}I-labelled albumin preparation and those published values, which are currently accepted, is the justification for subsequent use of the commercial preparation. Inspection of the widely varying published values demonstrates the importance of establishing a normal range in a particular laboratory or study.

The discrepancies in transfer rates between the present ones and those of Cohen *et al* (1961) are somewhat surprising. Evidence for acceptance of the current values is that the total exchange rate does not differ significantly from that given by Takeda and Reeve (1963), ie, k_1 calculated by their model system; they noted the difference from that given by Cohen *et al* (1961). Takeda and Reeve (1963) used autologous albumin prepared by salt fractionation and labelling was performed by the iodine monochloride technique. Beeken, Volwiler, Goldsworthy, Garby, Reynolds, Stogsdill, and Stemler (1962) also found the value for the content of the rapidly exchangeable pool in their normal series to be somewhat lower than that given by Cohen *et al* (1961). However, unlike the present finding, they derived a slightly larger value for the content of the slowly exchanging pool than did Cohen *et al* and they provide evidence that their preparation was somewhat denatured.

In the routine use of this preparation of iodinated albumin, as with all other preparations, whatever the source, it is essential to ensure that individual aliquots are satisfactory. In experimental groups emphasis was put on the excretion of isotope in the first 24-48 hours after injection, and, if steady-state conditions existed, on the constancy of the daily ratio of urine to serum radioactivity (see McFarlane, 1964, 1965). When two groups of subjects were compared, one member of each group received part of the same aliquot of isotope.

In comparing a group of patients with the control series it has to be remembered that Rossing (1967) found significant sex differences in albumin content and absolute catabolic rate and that Yan and Franks (1968) showed that the intravascular and interstitial contents of albumin were decreased in six healthy old subjects (aged 72-85 yr), although catabolic and transfer rates were unchanged relative to those of young subjects. Unfortunately, in this as in many other studies, it was impossible to obtain sufficient volunteers to establish normal sex and age grouped values.

One must conclude that this commercial preparation is satisfactory for use in metabolic studies on patients. Inspection of results obtained for albumin metabolism on over 50 patients (eg, Ballantyne and Fleck, 1973) indicates that no aliquot of the ^{125}I-labelled albumin showed evidence of gross denaturation.

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