SOME OBSERVATIONS ON THE METABOLISM OF ^{131}I-ALBUMIN IN CUSHING'S SYNDROME AND DURING ADRENOCORTICAL HORMONE ADMINISTRATION IN MAN

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In Cushing's syndrome excessive protein breakdown is thought to account for a large part of the clinical picture. Thus, loss of muscle power, thin skin, purple striae, easy bruising and osteoporosis have all been attributed to this process. In recent years albumin labelled with ^{131}I has been used as an indicator of stable albumin metabolism in the body. Despite early doubts about the validity of the technique, it has gained recognition today as a meaningful investigation, provided that conditions of radioactive labelling and preparation of albumin are standardized.[1-4] It must be stressed that the behaviour of this labelled compound is taken to reflect that of albumin in the body, and not necessarily of other protein moieties.

Sterling[5] reported the results of ^{131}I-albumin studies in 4 patients with Cushing's syndrome. He found an accelerated turnover-rate of albumin in the active disease; in addition, the total exchangeable albumin pool was decreased in comparison with normal controls. These changes were reversed by successful therapy of the disease. His study, then, appeared to substantiate the concept of increased albumin breakdown in the state of hyperadrenocorticism. But his data showed that the rate of degradation of albumin in absolute terms (grams per day or grams per day per square metre body surface) did not differ significantly from normal; because the albumin pool was smaller during the active phase of the disease, the turnover rate, expressed as a percentage of this pool, was higher.

We have studied 4 patients with Cushing's syndrome; in 3, studies have been repeated after treatment. Our method of analysis differs from that of Sterling, and our patients were investigated at an earlier stage after therapy. In addition, we report our findings in a case of congenital virilizing adrenogenital syndrome studied before and after prednisone administration. The effect of prednisone in a patient with Paget's disease of bone is also reported.

PATIENTS

Cushing's Syndrome

(*a*) Mrs. W., aged 33 years, presented with a 4½-year history of increasing obesity, weakness and tiredness, easy bruising and oligomenorrhoea. On examination she showed obesity of a cushingoid distribution, purple striae, multiple bruises and hypertension. X-rays showed osteoporosis. A left-sided adrenal adenoma was removed and recovery has been complete.

(*b*) R.P., an 18-year-old male, complained of marked weight gain over the preceding 2 years. He displayed many features of classical Cushing's syndrome, but was unusual in that he was *grossly* obese. The whole of his right adrenal gland was removed, and, 3 weeks later, 7/8 of his left adrenal gland. Both were markedly hyperplastic. He has made a good recovery, but requires maintenance hydrocortisone.

(*c*) S.W., a 30-year-old female, presented with hypertension of unexplained origin. She showed truncal obesity, purple striae and a rounded, plethoric facies. At laparotomy, a left-sided adrenal adenoma was removed, and she has made an excellent recovery.

(*d*) P.C., a 16-year-old male, presented with an 18-month history of weight increase. He had clinical features of classical Cushing's syndrome. Unilateral adrenalectomy was performed (the adrenal weighing 12 G.), and a course of deep X-ray therapy was given to the pituitary gland.

Follow-up studies have not yet been made.

Comment. In all 4 cases of Cushing's syndrome the diagnosis was confirmed by the appropriate biochemical tests (urinary excretion of 17-hydroxysteroids, plasma cortisol level, and response to high dosage of glucocorticoids, ACTH and metopirone). In two cases, adrenal adenomas were found; in the remaining 2, the adrenals were bilaterally enlarged at laparotomy. These 4 patients were operated on by Prof. J. H. Louw, Head of the Department of Surgery.

2. *Congenital Virilizing Adrenal Hyperplasia*

(*e*) F.N., a 16-year-old female, was admitted because of abnormal external genitalia which had been present since birth. On examination, the 'phallus' was enlarged and there was a single urogenital sinus. A 'female' nuclear pattern on buccal-cell examination and high 17-ketosteroid excretion confirmed the diagnosis of female pseudohermaphroditism caused by congenital virilizing adrenal hyperplasia. Prednisone treatment diminished the level of 17-ketosteroid excretion. After several months of treatment breasts developed and reparative plastic surgery was later carried out by Mr. D. Davies.

3. *Paget's Disease of Bone treated with Prednisone*

(*f*) D. le R., a 52-year-old male, was admitted with neurological complications of severe, generalized Paget's disease of

bone. The clinical and biochemical response to a 6-week trial period of prednisone did not justify continued treatment.

4. *Normal Controls*

These were selected from hospital inpatients, in whom there was no reason to suspect any abnormality of protein metabolism. The figures obtained for mean albumin pool-size and degradation rate may indicate a minor degree of protein depletion in some of these subjects: reasons for this are discussed elsewhere.[6]

METHODS

These have been reported in detail elsewhere.[6] In brief, pure human albumin was prepared by fractionation of plasma through a carboxy-methylcellulose column using an acetate buffer.[7] ^{131}I-iodination was achieved by the use of a potassium iodide/iodate mixture, which overcomes the effect of thiosulphate solution, in which ^{131}I is customarily supplied.[8] Free iodine was removed by passage through a Dowex resin column, the eluate showing not less than 98% of the ^{131}I to be protein-bound, when tested by trichloroacetic-acid precipitation. Carrier albumin was added to the final preparation to reduce radiation damage, and Seitz-filtration was performed to ensure sterility. Fresh preparations of ^{131}I-albumin were used for each experiment.

Solutions containing 10 - 20 μc. of ^{131}I were administered intravenously to subjects whose thyroid glands had been blocked by previous administration of Lugol's iodine or sodium iodide. A blood sample was taken at 10 minutes for calculation of plasma volume by the isotope-dilution method. Daily blood samples and 24-hour urine collections were assayed for radioactivity for a period of 7 - 10 days. A well-type scintillation counter (sodium iodide crystal) was used for this purpose, aliquots of urine and plasma being counted for sufficient time to achieve better than 2% statistical accuracy. Suitable radioactive standards were prepared at the time of administration of the tracer.

Stable plasma albumin was assayed in quadruplicate by the biuret method[9] at the beginning of, during, and at the end of each individual study, mean figures being used in calculations.

All data were analysed by the 'equilibrium-time' method.[5,10] This assumes that the tracer albumin is mainly distributed between two 'compartments' in the body, one corresponding to the blood plasma, the other being 'extravascular'. Fig. 1 represents this state of affairs diagrammatically.

The ^{131}I-albumin is introduced into the intravascular compartment, A, which is in equilibrium with the extravascular compartment, B. Degradation of albumin is assumed to occur within compartment A or in tissues which equilibrate rapidly with it. Excretion of the ^{131}I label takes place from compartment A only. Since uptake of free ^{131}I by the thyroid gland has been blocked by the administration of Lugol's iodine to

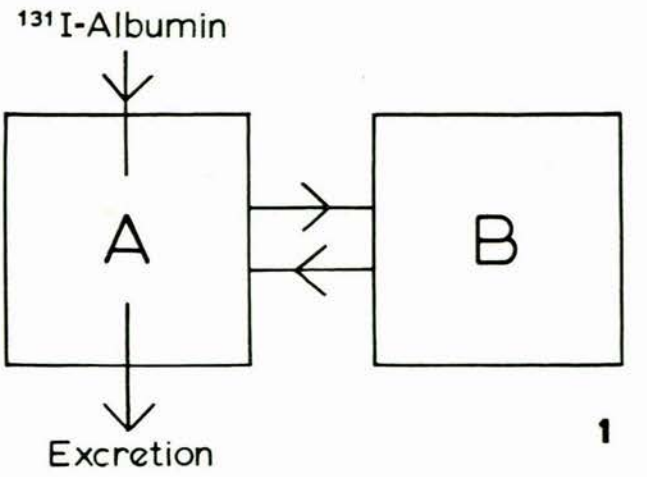

Fig. 1. A diagrammatic representation of an open two-compartment system (mammillary), in terms of which the kinetics of ^{131}I-albumin have been analysed (see text for explanation).

TABLE I. RESULTS OF INDIVIDUAL STUDIES

Condition	*Subject*	*Sex*	*Age*	*Status*	*Weight (kg.)*	*Serum albumin (G. %)*	*Half-time (days)*	*Turnover rate† (%/day)*	*Total albumin pool**	*Plasma albumin pool**	*Extra-vascular albumin pool**	*Degradation rate***
Cushing's syndrome	Mrs. M.W.	F	33	1. Disease active	102·8	4·36	6·0	11·6	300·1 (2·92)	115·3 (1·12)	184·8 (1·80)	13·4 (130)
				2. 1 month postop.	97·0	3·21	4·0	17·5	259·3 (2·67)	77·9 (0·80)	181·4 (1·87)	13·6 (141)
				3. 9 months postop.	84·1	4·54	6·9	10·0	280·7 (3·34)	108·3 (1·28)	172·4 (2·06)	10·8 (128)
Cushing's syndrome	R.P.	M	18	1. Disease active	96·6	4·21	6·4	10·8	229·8 (2·38)	88·9 (0·92)	140·9 (1·46)	9·6 (99)
				2. 2 months postop.	93·0	4·05	5·4	12·8	248·2 (2·67)	89·2 (0·96)	159·0 (1·71)	11·4 (123)
				3. 7 months postop.	83·0	4·28	5·9	11·8	292·4 (3·53)	95·2 (1·15)	197·2 (2·38)	11·2 (135)
Cushing's syndrome	S.W.	F	30	1. Disease active	65·0	4·28	6·9	10·0	274·2 (4·09)	106·2 (1·63)	168·0 (2·46)	10·6 (163)
				2. 4 months postop.	63·2	4·05	7·7	9·0	262·8 (4·17)	108·8 (1·72)	154·0 (2·45)	9·8 (155)
Cushing's syndrome	P.C.	M	16	Disease active	74·1	4·73	6·9	10·0	245·6 (3·31)	115·1 (1·55)	130·5 (1·76)	11·5 (155)
Adrenogenital syndrome	F.N.	F	16	1. Disease active	44·4	3·83	10·4	6·7	213·6 (4·81)	86·6 (1·95)	127·0 (2·86)	5·8 (131)
				2. 3 weeks of prednisone	45·5	4·32	8·2	8·5	198·0 (4·35)	83·5 (1·84)	114·5 (2·51)	7·1 (156)
Paget's disease	D. le R.	M	52	1. Disease active	56·6	3·91	5·8	11·9	239·6 (4·23)	90·0 (1·59)	149·6 (2·64)	10·7 (189)
				2. 6 weeks of prednisone	57·7	3·88	4·9	14·2	222·7 (3·86)	90·0 (1·56)	132·7 (2·30)	12·8 (221)
Normal controls (31 subjects)						3·92	7·8	8·9	272·6 (4·43)	98·8 (1·61)	173·8 (2·82)	8·7 (143)

* Total, plasma and extravascular albumin pool sizes are given in grams; in parentheses below these figures, these pool sizes are expressed as grams per kg. body weight.

** Degradation rate is given as grams of albumin per day; the lower figure in parenthesis refers to mg. of albumin per kg. body weight per day.

† The turnover rate is the percentage of the mean plasma albumin pool that is degraded per day.

the patient, excretion of ^{131}I is taken to represent all that is removed from the albumin molecules during degradation. Mathematical treatment of the data for plasma and urinary radioactivity gives information about the sizes of these albumin compartments or pools, and the rate at which albumin is being degraded in the body. It is assumed that the subjects are in a steady state during the course of each study, i.e. that rate of synthesis equals the rate of degradation.

RESULTS

Table I lists the findings in all patients; normal control data, derived from 31 studies, are included for purposes of comparison. Because of fluctuations in body weight, particularly after surgery for Cushing's syndrome, total albumin pool sizes and degradation rates have been expressed in terms of body weight, as well as in absolute terms. In Fig. 2 the estimated total, and intra- and extravascular pool sizes have been represented graphically.

In the patients with Cushing's syndrome, with the exception of the first postoperative study on Mrs. W., an increase in total albumin pool in relation to body

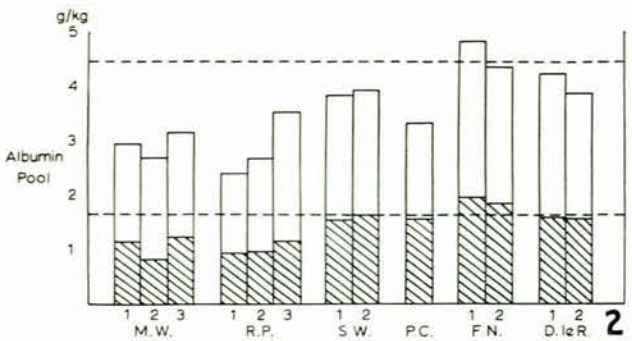

Fig. 2. Diagrammatic representation of albumin pool sizes (in G. per kg. body weight). Hatched and clear portions indicate plasma and extravascular pools respectively. The dotted lines indicate our mean normal control figures, derived from 31 studies. The numbers 1, 2 and 3, refer to successive studies in each patient (see Table I). M.W., R.P., S.W., and P.C. are the patients with Cushing's syndrome; F.N. is the patient with the adrenogenital syndrome; and D. le R. is the patient with Paget's disease.

weight is shown after treatment. Generally the pre-treatment total albumin pool (related to body weight) was significantly below our mean normal control figure; in 2 patients the post-treatment figures were still low, 7 and 9 months after therapy, when clinical evidence of Cushing's syndrome was no longer evident. In both prednisone-treated patients a fall in total albumin pool was noted. It can be seen that in all cases the extravascular albumin pool was predominantly affected.

No consistent change was found in turnover rate of albumin. Pre-treatment figures fell on either side of our normal range, and the changes produced by therapy were variable. This failure to find a change in albumin turnover rate occurred whether turnover was expressed in absolute terms or related to body weight.

DISCUSSION

For a valid application of the equilibrium-time method, subjects must be in a steady state. The discrepant result obtained in the early postoperative study on Mrs. W. may be explained by lack of fulfilment of this requirement. In the remaining cases the overall alterations during the period of study are so small that they are unlikely to detract from the validity of the technique.

Our results confirm those obtained by Sterling with regard to pool sizes, i.e. the total albumin pool, expressed in terms of body weight, is considerably lower than normal in active Cushing's syndrome, and this deficiency is repaired by successful treatment of the disease.

Our patient with virilizing adrenal hyperplasia showed a fairly large total albumin pool (possibly the result of prolonged androgenic anabolic action). Both she and the patient with Paget's disease showed diminution of this pool after prednisone treatment.

It is noteworthy that the extravascular protein pool is affected predominantly by alterations in adrenal status. In a study of protein-depleted and -repleted individuals[6] the extravascular pool was found to change earlier and to a greater extent than the plasma pool. It would appear that the body attempts to maintain preferentially the size of the plasma albumin pool in states of protein depletion.

From his data Sterling[5] has inferred that increased albumin synthesis, as well as degradation, results from excessive glucocorticoid action. Rothschild *et al.*[11] found similar changes in humans and rabbits treated with adrenocortical hormones. Attention has recently been drawn to the problems concerned with calculation of albumin synthesis rates in studies of this sort,[12] but a consideration of our data lends some slight support to Sterling's hypothesis. In the case of Mrs. W., for instance, the size of the total albumin pool, in absolute terms, was larger during the active phase of her disease when more albumin was being degraded; synthesis must, therefore, have been somewhat higher to maintain this larger pool in the face of a higher rate of degradation. Patient S.W. reflects the same sort of change to a lesser degree. In the two prednisone-treated patients there was a relatively greater increase in degradation rate during treatment than decrease in total albumin pool; a slight increase in albumin synthesis may be inferred. In the case of R.P., however, a considerable increase in total albumin pool followed therapy, despite a slight increase in degradation rate; this can only be explained by greater postoperative albumin synthesis, i.e. when hyperadrenocorticism was no longer present.

In summary, therefore, 4 of 5 patients showed changes consistent with the concept of increased albumin synthesis resulting from excessive glucocorticoid activity; the fifth patient showed contrary results. In view of this conflict, it would be wise not to draw firm conclusions regarding albumin synthesis until more information is available.

SUMMARY

^{131}I-albumin has been used to study stable albumin metabolism in 4 patients with active Cushing's syndrome; in 3, studies were repeated after surgical correction of the disease. A patient with congenital adrenogenital syndrome and one with Paget's disease of bone were studied before and after prednisone therapy.

In general, excessive glucocorticoid activity appears to be associated with a lowering of the total albumin pool of the body, if this is related to body weight. No significant change in degradation rate of albumin was found,

but there is slight evidence in favour of increased albumin synthesis under the influence of adrenocortical hormones.

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