

To what extent the increased disposal of lactate and F.F.A. in athletes during exercise is due to increases in the activities of rate-limiting enzymes or to other factors remains an open question.

We thank students of Glasgow and Oxford universities and members of the Garscube Harriers and Thames Valley Harriers for acting as subjects. This work was supported by the Royal Society, the Medical Research Council, the Medical Commission for Accident Prevention, the Peel Trust, and the U.S. Public Health Service (grant no. AMO8715). Prof. J. A. Simpson and Prof. J. N. Davidson have provided encouragement, and Dr. J. L. Corbett collaborated in early investigations. R. H. J. is in receipt of the E. G. Fearnside scholarship of the University of Cambridge, and D. H. W. is a member of the external staff of the Medical Research Council.

Requests for reprints should be addressed to R. H. J., Department of Neurology, Killearn Hospital, Killearn, by Glasgow.

REFERENCES

1. Courtice, F. C., Douglas, C. G. *Proc. R. Soc. B.* 1936, **119**, 381.
2. Cooper, K. E., Johnson, R. H., Pugh, L. G. C. E. Unpublished.
3. Pugh, L. G. C. E., Corbett, J. L., Johnson, R. H. *J. appl. Physiol.* 1967, **23**, 347.
4. Åstrand, P.-O., Hallback, I., Hedman, R., Saltin, B. *ibid.* 1963, **18**, 619.
5. Williamson, D. H., Mellanby, J., Krebs, H. A. *Biochem. J.* 1962, **82**, 90.
6. Hohorst, H. J., Kreutz, F. F., Bücher, T. *Biochem. Zeit.* 1959, **332**, 18.
7. Bergmeyer, H. U., Bernt, E. in *Methods of Enzymatic Analysis* (edited by H. U. Bergmeyer); p. 123. Weinheim, 1963.
8. Kreutz, F. *Klin. Wschr.* 1962, **40**, 362.
9. Itaya, K., Ui, M. *J. Lipid Res.* 1965, **6**, 16.
10. Holmgren, A. *Scand. J. clin. Lab. Invest.* 1956, **8**, suppl. no. 24, p. 1.
11. Holmgren, A., Strom, G. *Acta med. scand.* 1959, **163**, 185.
12. Cobb, L. A., Johnson, W. P. *J. clin. Invest.* 1963, **42**, 800.
13. Juchems, R., Kumper, E. *New Engl. J. Med.* 1968, **278**, 913.
14. Nelson, N., Grayman, I., Mirsky, I. A. *J. biol. Chem.* 1941, **140**, 361.
15. Bates, M. W., Krebs, H. A., Williamson, D. H. *Biochem. J.* 1968, **110**, 655.
16. Björntorp, P., Scherstén, T. *Am. J. Physiol.* 1967, **212**, 683.
17. Balasse, E., Ooms, H. A. *Diabetologia*, 1968, **4**, 133.
18. Senior, B., Loidan, L. *Nature, Lond.* 1968, **219**, 83.

REAGINIC ANTIBODY (IgE), SKIN, AND PROVOCATION TESTS TO DERMATOPHAGOIDES CULINÆ AND HOUSE DUST IN RESPIRATORY ALLERGY

BRITA STENIUS

Department of Pulmonary Diseases,
University Central Hospital, Helsinki, Finland

LEIF WIDE

Department of Clinical Chemistry,
University Hospital, Uppsala, Sweden

Summary Circulating reaginic, IgE, antibody was found in patients with respiratory allergy to specific allergens in extracts of house dust and of *Dermatophagoides culinæ*, and less so to its culture medium. Skin and provocation tests to house dust and *D. culinæ* correlated well with their respective specific IgE antibody, though there were no significant correlations between house dust and *D. culinæ* extracts in the IgE and skin test reactions. There was partial inhibition of IgE reactions to the *D. culinæ* extract, but not vice versa. The extracts of house dust and *D. culinæ* had some common as well as unrelated antigens.

Introduction

THE role of mites as possible allergens in house dust has been intensively investigated lately, in particular by Voorhorst et al.¹ who reported that the acarine mite

Dermatophagoides pteronyssinus produces an allergen which is identical with the one in house dust. Other mites of genus *Dermatophagoides*, *D. culinæ* and *D. farinae*, have been investigated in Britain and Japan and found to be sources of clinically important allergens resembling those in house dust. All these mites have been found in house-dust samples. The different species of *Dermatophagoides* have given similar skin-test results and seem to have related allergens. A few other mites such as *Glycyphagus* sp. and *Acarus siro* have also been investigated and found to play an important though lesser part in house-dust allergy.

Only recently has it been possible to demonstrate in vitro the antibody of the anaphylactic or immediate type of allergy, the reaginic antibody IgE. The radio-allergosorbent test (R.A.S.T.)² makes it possible to determine the amount of circulating reaginic antibodies to specific allergens.

We have tried to clarify the following questions: are there circulating IgE antibodies to *D. culinæ* allergen? If so, do they show any correlation with skin-test reactions to this allergen; to what extent are the allergens in the *D. culinæ* extract identical with or related to those in a commercial house-dust extract; and does the growth medium influence the allergenicity of the mite extract?

Materials and Methods

A group of 33 patients with either asthma or allergic rhinitis and with known or suspected allergy to house dust was investigated. The patients were questioned for history of house-dust sensitivity.

The allergen solutions used were house-dust extract, (Meda, Dome) 10,000 protein-nitrogen units, and carbol-saline Seitz-filtered dialysed freeze-dried extracts of a culture of *Dermatophagoides culinæ* and of the constituents of the medium on which it was grown. The medium consisted of Gaines dog-meal which is prepared from wheat, maize, soya bean, bone and fish meal, meat byproducts, animal fats, and added vitamins. A pinch of yeast, *Sacharomycetis cerevisiae*, is added to the culture medium. (These materials were provided by Miss V. Holford-Strevens of the department of clinical immunology, Institute of Diseases of the Chest, London.) Carbol saline and histamine solutions were used as controls in the skin testing.

Skin tests were done by the prick method and were considered unequivocally positive if the weal diameter was 2 mm. or more. A weal diameter of 1 mm. was considered doubtful. Nasal and inhalation tests were done in some cases.

The reaginic antibodies (IgE) in serum were semi-quantitatively estimated by R.A.S.T.,² with a few modifications. For the preparation of the polymer-allergen conjugates 100 mg. of the dried extracts of the mites and the medium constituents were dissolved in 1 ml. of 0.075M phosphate-buffered saline solution of pH 7.4 and incubated with 100 mg. of cyanogen-bromide-activated 'Sephadex' at 4°C for three days. The 0.1M trometamol ('tris') buffer of pH 7.4 previously used in the assay procedure was replaced by a 0.075M phosphate-buffered saline solution of the same pH. For the calibration of the second step of the R.A.S.T. reaction, where the labelled anti-IgE antibodies were added, the uptake of radioactivity on known amounts of an immunosorbent consisting of IgE coupled to sephadex was determined each time. The degree of positive reactivity in the R.A.S.T. was expressed in 'sorbent units' (s.u.) where 1 s.u. is the radioactivity uptake on the immunosorbent to which 1 pg. of IgE was coupled. The basis for this method of expressing the reactivity in the R.A.S.T. will be discussed in detail elsewhere.

TABLE I—PRICK-TEST AND R.A.S.T. RESULTS

Patient	History of allergic reaction to house dust	Results of prick tests (mm. diameter) and R.A.S.T. (graded 0-5) to:								Provocation test with	
		Commercial house dust		<i>D. culinae</i>		Yeast		Gaines 'Gadog-meal'			
		P	R	P	R	P	R	P	R	<i>D. culinae</i> * (1 mg./ml.)	House-dust inhalation
1	—	3	0	1	0	1	0	0	0	..	..
2	—	4	2	4	0	0	0	0	2	—	..
3	+	5	4	4	2	0	0	4	2	+†	..
4	—	0	0	2	2	0	0	0	0	..	..
5	—	0	1	0	0	0	0	0	0	..	..
6	+	3	0	3	0	0	0	0	0	—	—
7	+	0	0	0	0	0	0	0	2	..	..
8	+	2	2	3	2	2	2	4	3	..	..
9	+	0	0	2	0	0	0	0	0	—	..
10	+	5	3	7	3	3	2	0	1	+	..
11	—	1	2	2	0	0	0	0	0	—	..
12	+	0	0	3	1	0	0	1	0	—	..
13	—	3	1	4	2	0	1	1	0	..	—
14	—	2	1	4	1	0	0	0	0	—	—
15	+	4	2	5	2	2	1	1	1	..	+
16	—	6	3	0	0	0	0	0	0	..	+
17	—	4	3	4	2	0	0	0	0	..	+
18	—	3	2	2	1	1	0	4	1	..	..
19	+	2	3	0	0	0	0	0	0	..	..
20	+	7	3	5	3	4	2	5	1	+	..
21	+	3	2	0	0	0	0	3	2	..	..
22	+	5	3	4	2	0	0	0	0	—†	+
23	—	3	0	3	2	4	0	5	3	..	—
24	—	5	2	6	4	3	0	3	0	..	..
25	+	3	3	2	2	7	4	4	2	..	..
26	—	3	0	7	0	5	4	4	0	..	..
27	+	2	2	7	3	7	3	4	2	..	..
28	+	3	3	7	5	2	0	1	0	..	..
29	+	2	4	0	0	0	0	0	0	..	..
30	+	0	0	0	0	0	0	2	0	..	..
31	—	2	2	2	1	3	3	0	1	..	..
32	+	2	2	6	5	2	1	2	1	..	..
33	+	0	0	1	0	3	3	1	1	..	..
Pearsons correlation coefficient (r)		0.613 (P<0.001)		0.765 (P<0.001)		0.853 (P<0.001)		0.618 (P<0.001)		..	..

* By inhalation in patients 2, 3, 6, 10, 11, and 22; nasally in patients 9, 12, 14, and 20. † 10 mg. per ml.
Prick test and R.A.S.T. were concordant (both positive or both negative) in 26/33 (79%) of patients tested with commercial house dust and 24/33 (79%) of patients tested with *D. culinae* extract.

TABLE II—RELATION BETWEEN HOUSE DUST AND *D. culinae* EXTRACT REACTION IN PRICK AND R.A.S.T. TEST

<i>D. culinae</i>	Prick test*		R.A.S.T.†	
	+	—	+	—
+	20	4	12	3
—	5	4	8	10
*r=0.437 (P<0.05)			†r=0.232 (P<0.2)	

The R.A.S.T. reaction was considered positive when the radioactivity uptake was twice that of the negative controls, and negative when the uptake was less than 1.5 times the control values. The values between positive and negative reactions were regarded as "doubtful".

Positive reactions varied between 0.7 and 1000 s.u. per µl. serum. For the comparison between the results of the R.A.S.T. and the prick tests, the R.A.S.T. reactions were divided into six groups: (0) negative; (1) "doubtful"; (2) from about 0.7 to 5 s.u. per µl.; (3) from 5.1 to 30 s.u. per µl.; (4) from 30.1 to 200 s.u. per µl.; and (5) more than 200 s.u. per µl. (table I).

The inhibition tests were made by preincubation of 20 µl. of serum with 20 µl. of the allergen solution (100 mg. per ml.) for 20 hours before assay with the R.A.S.T. and the results were compared with those obtained when the pre-incubation was made with saline solution.

The statistical analysis was carried out in two ways: (1) Pearsons correlation coefficient (r) was calculated taking into consideration the actual values for reaction sizes; (2) the percentage of agreement between positive and negative results with the skin test and the R.A.S.T. was calculated.

Unless stated otherwise, the "doubtful" reactions were included in the negative groups.

Results

Demonstration of Reaginic Antibody (IgE) in Vitro to *D. culinae*

Reaginic antibodies of the IgE type to *D. culinae* extract were shown in 20 patients (table I).

Relationship between the Prick-test Results and R.A.S.T. Results

Prick test and R.A.S.T. were concordant (both positive or both negative) in 79% of house-dust tests and in 73% of tests with *D. culinae* extract. Depending on the antigen, the correlation coefficient (r) between skin test and R.A.S.T. varied from 0.613 to 0.853, and in all cases it was statistically significant (P<0.001). In those cases where the two results were discordant the prick test was usually positive and the R.A.S.T. negative.

Inhalation or nasal tests with *D. culinae* extract were done in 6 of the 9 cases with positive prick test but negative R.A.S.T., and with house dust in 4 of the 6 cases with positive prick test but negative R.A.S.T. In all these cases the provocation test was negative. In the patients with a positive prick and R.A.S.T. test, 3 out of 4 gave positive provocation tests to *D. culinae* extract and 4 out of 4 to house-dust extract.

Relation between House-dust and *D. culinae* Extracts

No significant correlation was found between the reactions to the commercial house-dust extract and the

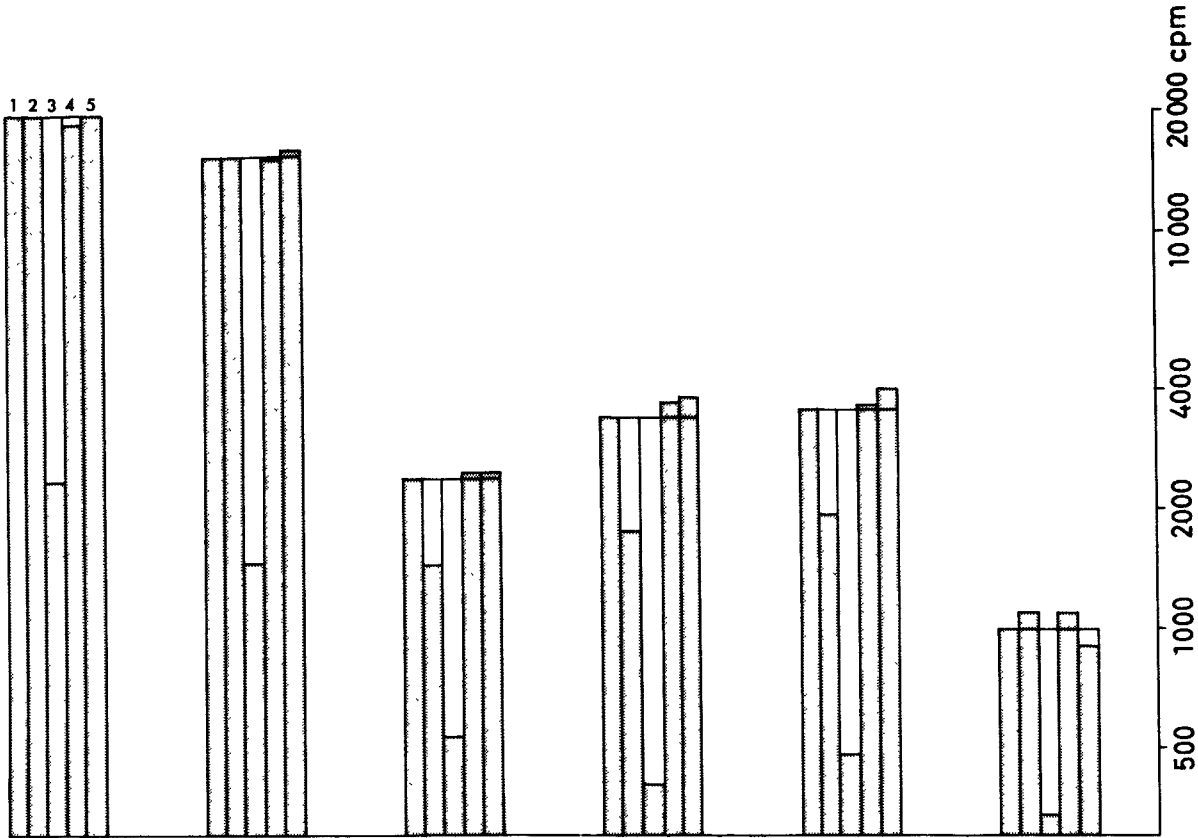


Fig. 1—Inhibition tests on sera giving positive R.A.S.T. reactions to the *D. culinae* extract.

R.A.S.T.s were done after incubation of serum with one of the following:
(1) Saline solution. (2) House dust. (3) *D. culinae*. (4) Dried yeast. (5) Gaines dog-meal.
The patients are, left to right, nos. 28, 32, 10, 24, 27, and 17.

reactions to the *D. culinae* extract. This was so both by skin testing and by R.A.S.T. (table II).

Reactions to Culture Medium

Positive R.A.S.T. reactions were found to the yeast extract and to the dog-meal extract in 8 cases each, and of these 3 reacted to both. Positive prick-test reaction to the yeast extracts were found in 13 cases and to the dog meal in 12 cases, and of these 8 reacted to both.

R.A.S.T. Inhibition Tests

In 6 cases the R.A.S.T. reaction to *D. culinae* was inhibited by *D. culinae* extract (fig. 1). In 3 of these the

D. culinae reaction was partially inhibited by the house-dust extract, but there was no inhibition in the other 3.

In 3 cases with a positive R.A.S.T. reaction to house dust there was no inhibition of the reaction with *D. culinae* extract (fig. 2). It is notable that 2 of these patients' showed strongly positive R.A.S.T. reactions to the *D. culinae* extract as well. None of the reactions mentioned above were inhibited by the yeast or dog-meal extracts.

Inhibition of the reaction to the yeast extract was attempted in 2 cases (fig. 3). Both were inhibited by the yeast extract and to a lesser extent by *D. culinae* extract but not by the extracts of house dust or dog meal.

Discussion

The R.A.S.T. results confirm that antibodies of the IgE type to *D. culinae* extract are present in the sera of

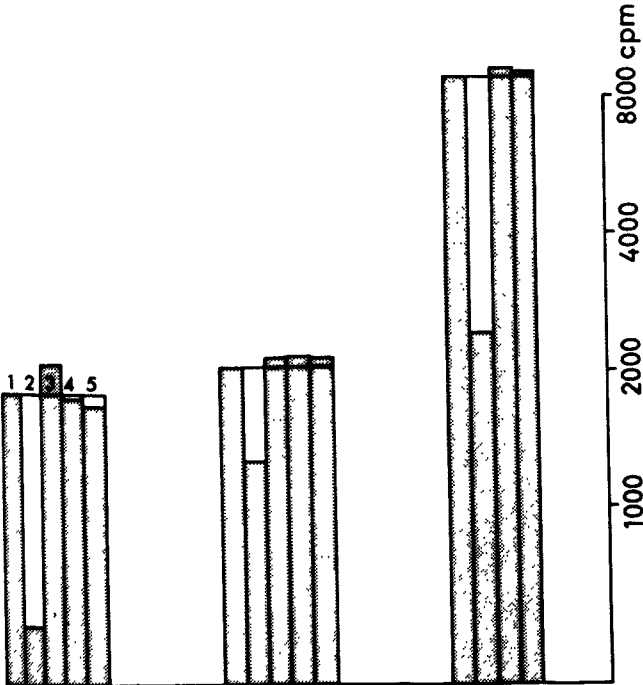


Fig. 2—Inhibition tests on sera giving positive R.A.S.T. reactions to the commercial house-dust extract.
The patients are, left to right, nos. 10, 18, and 3.
(Key as in fig. 1.)

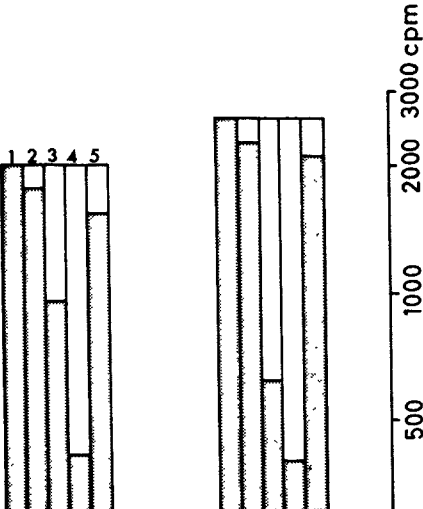


Fig. 3—Inhibition tests on sera giving positive R.A.S.T. reactions to the yeast extract.
Patients are, left to right, nos. 10 and 27.
(Key as in fig. 1.)

some atopic patients. This was expected since several investigators have found, by skin and provocation tests, that *Dermatophagoides* sp. are sources of important allergens.

Wide et al.² reported correlations of about 68% between intracutaneous tests and R.A.S.T. and 96% between inhalation tests and R.A.S.T. We found a correlation of 73% between R.A.S.T. and skin-prick tests with *D. culinae* extract. If the reactions regarded as doubtful were added to the positives, the figure would be 82%.

Where prick-test and R.A.S.T. results were discordant, the skin test was usually positive and the R.A.S.T. negative, and the negative R.A.S.T. results corresponded with negative provocation tests. This confirms the not uncommon observation that skin tests may give positive results which seem to be clinically irrelevant.

The fact that we could not correlate the *D. culinae* reaction with the commercial house-dust extract reaction was unexpected since a correlation with other house-dust extracts has been found by others. Dutch, Japanese, and British workers have all concluded that the *Dermatophagoides* spp. are probably the most important allergens in house dust.^{1 3-6} Most of these investigations, however, used different house-dust extracts from those used by us. Morrow-Brown⁶ also using a 'Dome' house-dust extract came to the conclusion that *D. culinae* was an important allergen in house dust. One of us⁷ has also found a statistically highly significant correlation between the *D. culinae* extract and a commercial house-dust extract prepared by Bencard. Miyamoto et al.³ in inhibition tests by the passive transfer method obtained inhibition of the *D. farinae* reaction with a house-dust extract and vice versa. *D. farinae* and *D. culinae* are regarded as identical species.⁸

It is very likely that different house-dust extracts will vary as to their contents of *Dermatophagoides* sp. allergen. In several patients vigorous reactions were obtained to house-dust extract and none to the *D. culinae*, and vice versa. The inhibition tests confirm that there are allergens in the house-dust extract that are not present in *D. culinae* and vice versa. On the other hand, the inhibition tests also show that they have related or identical allergens. The house-dust extract investigated here may contain allergens from other mites to which our population may have been sensitised. The mite flora in Scandinavia has not so far been thoroughly investigated.

We found more positive prick-test reactions to the growth-medium extracts than have previous investigators, and these were supported by the R.A.S.T. test. Pepys et al.⁴ reported that 2 out of 27 patients had prick-test reactions to the dog-meal extract, and 5 out of 22 to the yeast extracts. Our figures are 12 out of 33 and 13 out of 33, respectively. This suggests that allergy to the constituents of the dog-meal and yeast extracts is more frequent in Finland and Sweden.

The inhibition of the yeast reaction by the *D. culinae* extract indicates that the two extracts had some allergens in common. The possibility that some of the positive *D. culinae* reactions may have been due to contamination with the culture medium must be considered. In most cases, however, the positive *D. culinae* reactions were elicited by specific antigenic

determinants that were not present in the culture medium.

The poor correlation between the history of house-dust allergy with both skin tests and R.A.S.T. differs from the highly significant correlation between the history and skin tests observed in the U.K.⁴ and elsewhere. This striking difference requires further investigation.

We thank Miss Gun Almqvist for technical assistance; Pharmacia AB for supply of special sephadex preparations; Dr. Gunnar Dahlstrom, for giving us access to patients and work facilities in his clinic, and his staff, especially Dr. Karin Osterman for doing the skin and provocation tests; Prof. Jack Pepys, for helpful discussions; and Miss Valerie Holford Strevens for preparing the antigens. This work was supported by grants from the Finnish Anti-Tuberculosis Association and the Swedish Medical Research Council (16-637).

Requests for reprints should be addressed to B. S.

REFERENCES

1. Voorhorst, R., Spijksma, F. T. M. *J. Allergy*, 1967, **40**, 357.
2. Wide, L., Bennich, H., Johansson, S.-G. *Lancet*, 1967, **ii**, 1105.
3. Miyamoto, T., Oshima, S., Ishizaka, T., Sato, S. *J. Allergy*, 1968, **42**, 1.
4. Pepys, J., Chan, M., Hargreave, F. E. *Lancet*, 1968, **i**, 1970.
5. Maunsell, K., Wraith, D. G., Cunningham, A. M. *ibid.* p. 1267.
6. Brown, H. M. *Br. med. J.* 1968, **iii**, 646.
7. Stenius, B. *Duodecim*, 1969, **85**, 283; *Scand. J. resp. Dis.* (in the press).
8. Spijksma, F. T. M. Personal communication.

ALDOSTERONE AND SODIUM RESPONSE TO LITHIUM ADMINISTRATION IN MAN

DENNIS L. MURPHY FREDERICK K. GOODWIN
WILLIAM E. BUNNEY, Jr.

*Section on Psychiatry, Laboratory of Clinical Science,
National Institute of Mental Health, Bethesda,
Maryland 20014*

Summary Administration of lithium carbonate to 16 manic-depressive and depressed patients was found to lead to an initial 1-2 days of sodium and water diuresis. In the subsequent 4-5-day period of treatment, sodium retention associated with a 50% increase in aldosterone excretion occurred, followed in the next several days by a return towards pre-lithium levels. These changes occurred in all patients and were apparently not related to the initial clinical state of the patient nor the presence or absence of symptomatic improvement with lithium.

Introduction

LITHIUM carbonate has proved effective in the treatment of mania in each of three double-blind studies,¹⁻³ as well as in most other clinical trials.⁴ Lithium's chemical relationship to sodium and its ability to substitute for sodium in some biological systems⁵ have generated interest in its effects on sodium metabolism. Administration of lithium to man has been found to alter urinary sodium excretion and lead to sodium retention⁶⁻⁸; but two isotope dilution studies of the effects of lithium on exchangeable ²⁴Na yielded contradictory results.^{9 10} Changes in total body-water and intracellular water occur after lithium,¹⁰ but urinary potassium excretion is little affected.^{7 9} In the present study the possible involvement of aldosterone in the changes in sodium balance accom-