circulating hormone is inactive. The substance which inactivates the circulating insulin in acromegalic patients may be similar to, if not identical with, the insulin inhibitor extracted from normal plasma by Vallance-Owen et al. (1958a and b); this substance is not present in the plasma of hypophysectomised patients, and its inhibitory action is disproportionately reduced by dilution. Thus the abnormal insulin effects produced by diluted plasma from male acromegalics could be due to the presence in undiluted plasma of large amounts of an insulin inhibitor whose effects are abolished by plasma dilution; no such inhibitor has yet been isolated or identified. The abnormal amount of insulin present in undiluted plasma may be necessary to compensate for the abnormal resistance of otherwise sensitive peripheral tissues. This hypothesis, though it may account for the high levels of activity observed in male subjects, does not explain why undiluted and diluted plasma from comparable female [illegible] have essentially normal levels of activity.

[illegible] (1956) has shown that the rat-diaphragm method of insulin assay is "sensitive but not very precise"—an assessment with which Willebrands and Groen (1958) agree. Concentrations of the order of 100 micro-units per ml. were easily detected in the present experiments, though in lower concentrations (10 micro-units per ml.) insulin did not always produce significant effects in individual experiments. The insulin equivalent values deduced from the effects produced by individual plasma specimens must be regarded as only approximate: the correct value may be anything from a half to twice the estimated figure. Thus, though this method of assay is very sensitive, its inaccuracy may be responsible for its failure to provide information of clinical significance in individual cases. There is considerable evidence (Groen et al. 1952, Randle 1954c, Vallance-Owen and Hurlock 1954, Randle and Taylor 1958) that the biological effects produced by plasma are due to the insulin which it contains, but other substances may also be present which augment or diminish its effects. Further investigations, similar to those of Field and Stetten (1956) and Vallance-Owen et al. (1958a and b) and aimed at the detection and identification of such inhibitors and augmentors in plasma, may yield more useful information concerning abnormal carbohydrate metabolism in human disease than the less specific plasma-insulin assays carried out in the past.

Summary

Insulin assays were carried out on plasma drawn from 18 normal subjects, 14 acromegalics, 7 patients with islet-cell tumours of the pancreas, and 3 children with idiopathic spontaneous hypoglycæmia.

Normal undiluted plasma has a biological effect equivalent to that of insulin in a concentration of approximately 70 micro-units per ml. The insulin equivalence of plasma diluted at least 4-fold is about 200 micro-units per ml.

Plasma from female acromegalics, assayed in the undiluted and diluted form, has essentially normal biological activity, while at all levels of dilution plasma from male acromegalics is significantly more active. It is suggested that the latter contains high concentrations of an insulin inhibitor.

Plasma from 3 of the cases of islet-cell tumours was abnormally active. It is concluded that normal levels of activity in suspected cases do not contraindicate this diagnosis.

Normal levels of activity were found in the 3 cases of spontaneous hypoglycæmia of infancy.

Whereas knowledge of plasma-insulin activity may help to explain the underlying causes of abnormal carbohydrate metabolism in some pathological conditions assays in individual cases seldom provide information of use to the clinician.

I should like to thank those clinicians of other hospitals who kindly supplied me with plasma from the cases of spontaneous hypoglycæmia; Dr. P. M. Bishop, Prof. W. J. H. Butterfield, and Dr. J. H. Briggs for their cooperation in the study of acromegalic patients carried out in this hospital; Prof. R. H. S. Thompson for his encouragement and advice; and Mr. K. G. Kilbourn for his skilled technical assistance.

REFERENCES

Fornstein, J., Lawrence, R. D. (1951) *Brit. med. J.* i, 732.
Cochrane, W. A., Payne, W. W., Simpkiss, M. J., Woolf, L. I. (1956) *J. clin. Invest.* 35, 411.
Conn, J. W., Seltzer, H. S. (1955) *Amer. J. Med.* 19, 460.
Field, J. B., Stetten, De W. (1956) *Diabetes*, 5, 391.
Groen, J., Kamminga, C. E., Willebrands, A. F., Blickman, J. R. (1952) *J. clin. Invest.* 31, 97.
Hardwick, C., Butterfield, W. J. H., Kelsey Fry, I., Briggs, J. H. (1959) *Proc. R. Soc. Med.* 52, 807.
Himsworth, H. P., Kerr, R. B. (1939) *Clin. Sci.* 4, 119.
Kelly, J. D. C. (1956) *Lancet*, i, 668.
Kinsbourne, M., Woolf, L. I. (1959) *Arch. Dis. Childh.* 34, 166.
Lawrence, R. D. (1951) *Brit. med. J.* i, 373.
McQuarrie, I. (1954) *Amer. J. Dis. Childh.* 87, 399.
Martin, D. B., Renold, A. E., Dagenais, Y. M. (1958) *Lancet*, ii, 76.
Randle, P. J. (1954a) *ibid.* i, 441.
— (1954b) *ibid.* p. 899.
— (1954c) *Brit. med. J.* i, 1237.
— (1955) The Hypophyseal Growth Hormone, Nature and Actions p. 143. New York.
— (1956) *J. Endocrin.* 14, 82.
— (1957a) *in* Ciba Foundation Colloquia on Endocrinology (edited by G. E. W. Wolstenholme and E. C. P. Millar); vol. 11, p. 115. London
— (1957b) *J. Endocrin.* 15, xxxix P.
— Taylor, K. W. (1958) *ibid.* 17, 387.
— Young, F. G. (1956) *ibid.* 13, 335.
Takeuchi, S., Ohashi, S., Kobayashi, Y. (1957) *J. Pharmacol.* 119, 436.
Vallance-Owen, J., Dennes, E., Campbell, P. N. (1958a) *Lancet*, ii, 336.
— — — (1958b) *ibid.* p. 696.
— Hurlock, B. (1954) *ibid.* i, 68.
— — Please, N. W. (1954) *ibid.* p. 983.
— — — (1955) *ibid.* ii, 583.
Whipple, A. O. (1952) *Canad. med. Ass. J.* 66, 334.
Willebrands, A. F., van der Geld, H., Groen, J. (1958) *Diabetes*, 7, 119.
— Groen, J. (1956) *ibid.* 5, 378.
Wright, P. H. (1957) *Lancet*, ii, 621.

LEUCINE AND PELLAGRA

C. GOPALAN
M.D. Madras, Ph.D. Lond.

S. G. SRIKANTIA
M.B., B.Sc. Mysore

From the Nutrition Research Laboratories, Indian Council of Medical Research, Tarnaka, Hyderabad 7, India

PELLAGRA has long been known to be predominantly a disease of maize-eaters. The association of pellagra with maize consumption has been attributed, among other factors, to the low tryptophan content of maize, and to the poor availability of its nicotinic acid. The disease is rare in areas where rice constitutes the staple. In nearly ten years' experience with many cases of nutritional deficiencies, we have seen only 4 classical cases of pellagra among the rice-eating population of Coonoor.

On the other hand, in Hyderabad, a city nearly five hundred miles further north, we frequently encounter cases of pellagra. Careful examinations of the dietaries of the poor segments of the population in Coonoor and Hyderabad failed to reveal any striking differences with regard to the intake of different nutrients; but, whereas rice is the sole staple in Coonoor, the dietaries of the poor segments of the population in Hyderabad invariably include the millet *Sorghum vulgare* (jowar). In practically every case of pellagra investigated by us in Hyderabad, a history of regular consumption of jowar with or without

AMINOACID COMPOSITION OF MAIZE, JOWAR, AND RICE

—	Tryptophan‡	Leucine‡	Isoleucine‡	Nicotinic acid§
Maize* ..	0·8	14·9	6·4	1·4
Jowar†	1·2	12·9	6·1	1·8
Rice†	1·2	8·0	6·0	1·2

* Baumgarten et al. (1946). ‡ g. per 100 g. protein.
† Balasubramanian et al. (1952). § mg. per 100 ml.

rice was obtained. In only 1 case was there a history of consumption of maize in addition to jowar and rice.

Comparison of the chemical composition of rice, jowar, and maize (see accompanying table) shows that the nicotinic-acid content of jowar is similar to that of rice. The reported tryptophan content of different strains of jowar varies widely, certain strains having nearly as high a content of the aminoacid as is found in rice while certain others have low values as in maize (Carpenter and Kodicek 1950). Both jowar and maize have, however, one common feature with regard to their aminoacid composition—namely, a high content of leucine. Elvehjem (1956) reported that the dietary supplementation of leucine at 1% level caused retardation of growth in rats subsisting on low-protein diets (9% casein), but that such growth retardation was not observed if the dietary protein intake was raised to 18%. The average daily protein intake in the dietaries of the pellagrins investigated here was of the order of 45 g. (9% protein), the protein being mainly derived from cereals including jowar. The possible role of aminoacid imbalance resulting from relative excess of leucine in the pathogenesis of pellagra

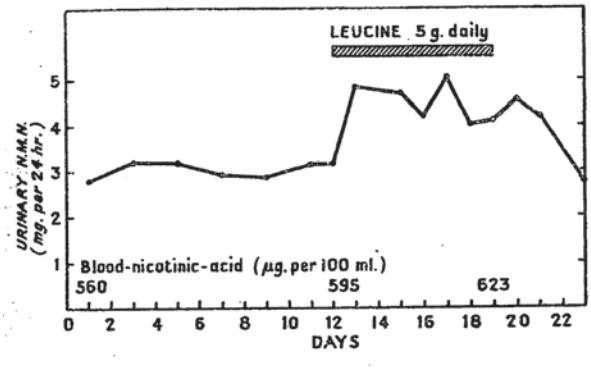

Fig. 1—Effect of supplementation with leucine on urinary N.M.N. excretion in a healthy subject.

was investigated, and the preliminary results are described here.

Experimental

Effect of Administration of Leucine on Urinary Excretion of N-methyl Nicotinamide (N.M.N.) in Healthy Volunteers and in Pellagrins

2 normal healthy male subjects were put on a basal diet providing roughly 10% protein, and their urinary N.M.N. excretion was determined daily by the method of Carpenter and Kodicek (1950). The basal diet was continued until a fairly stable level of N.M.N. excretion was attained. The volunteers were then given a daily supplement of 5 g. L-leucine along with one meal in a single dose, for seven days, and their urinary N.M.N. excretion was determined daily. Blood-nicotinic acid levels were determined by the method of Sweeney and Hall (1951) (with some modifications) before, during, and after leucine supplementation.

Similar studies were also carried out in 3 patients with pellagra.

To determine whether the changes in urinary N.M.N. excretion brought about by leucine administration could be influenced by isoleucine, 2 other healthy male subjects were investigated. These subjects also received 5 g. leucine daily for three days after the initial stabilisation period, but for four days thereafter they received 2 g. of DL-isoleucine daily in addition to the 5 g. L-leucine.

To facilitate the quantitative interpretation of the changes in N.M.N. excretion in terms of nicotinic-acid metabolism, two healthy volunteers, who had been earlier investigated with leucine as above, received a 15 mg. oral dose of nicotinic acid and the increase in their urinary excretion of N.M.N. was determined.

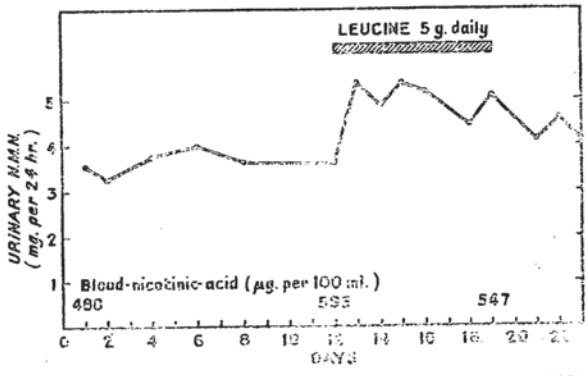

Fig. 2—Effect of leucine supplementation on urinary N.M.N. excretion in another healthy subject.

Effect of Isocaloric and Isonitrogenous Substitution of Rice by Jowar on Urinary N-methyl Nicotinamide Excretion

3 pellagrins were put on a basal diet providing 10% protein derived from rice and wheat, and the daily urinary N.M.N. excretion was determined. After the N.M.N. excretion had stabilised, rice in the diet was replaced by jowar and the daily urinary N.M.N. excretion on this diet was studied. Actual analysis of the two diets revealed that the rice-wheat diet in the 3 cases provided 7·3, 7·9, and 7·6 mg. nicotinic acid daily, while the jowar-wheat diet provided 7·8, 8·1, and 7·5 mg. nicotinic acid daily.

Effect of Administration of Large Amounts of Leucine in Pellagrins

2 patients with pellagra were placed on a basal diet supplying 45 g. of protein daily, the protein being mainly derived from jowar. After a preliminary stabilisation period these patients were given 10 g. L-leucine—twice daily in 1 case and thrice daily in the other—along with their principal meals. The urinary N.M.N. excretion was studied daily, and their clinical condition was carefully watched.

In this study the cases of pellagra were chronic, and the patients were not seriously ill. The patients volunteered for the investigation. We were confident that any deleterious effect of administering leucine could be corrected effectively and promptly by administration of nicotinic acid.

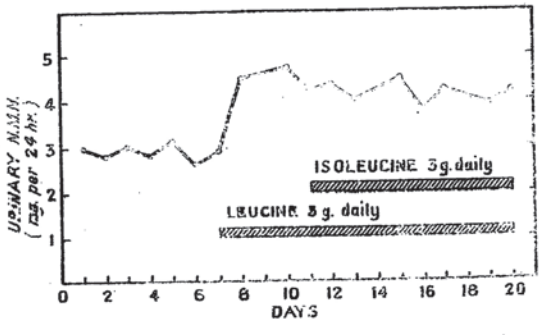

Fig. 3—Effect of simultaneous supplementation with leucine and isoleucine on urinary N.M.N. excretion.

Results

Effect of Administration of Leucine on Urinary Excretion of N.M.N.

The results of this study are illustrated in figs. 1–3. In all subjects investigated, both healthy volunteers and cases of pellagra, urinary excretion of N.M.N. increased immediately after administration of leucine. The rise in N.M.N. excretion brought about by 5 g. L-leucine daily represented an increase of nearly 50% over the basal level. Within a few days after the withdrawal of leucine, N.M.N. excretion returned to the basal level in all cases. There were no significant changes in the blood-nicotinic-acid levels. Determination of blood-nicotinic-acid levels in the same individual on a standard diet showed wide fluctuations, the values ranging from 660 µg. per 100 ml. to 1000 µg. per ml.

The increase in N.M.N. excretion brought about by leucine did not appear to be influenced by simultaneous administration of isoleucine (fig. 3). The administration of isoleucine did not alter the N.M.N. excretion pattern.

In the 2 healthy volunteers in whom the changes in N.M.N. excretion after administration of nicotinic acid were also studied, it was found that the administration of 15 mg. nicotinic acid resulted in an increase of urinary N.M.N. excretion of the order of 8·3 mg. and 7·5 mg. This suggests that the average increase of 1·7 mg. in N.M.N. excretion brought about by the administration of 5 g. of L-leucine reflects approximately an additional 3·5 mg. of nicotinic acid metabolised daily.

Effect of Isocaloric and Isonitrogenous Substitution of Rice by Jowar on Urinary N.M.N. Excretions

The effect of substituting jowar for rice in a typical case is illustrated in fig. 4. The replacement of rice by

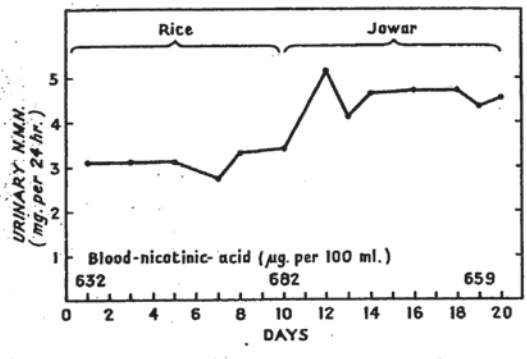

Fig. 4 Effect of replacing rice with jowar on urinary N.M.N. excretion in a patient with pellagra.

jowar resulted in a prompt increase in urinary N.M.N. excretion over the basal level observed in the "rice period". This increase was maintained as long as jowar was continued. After the withdrawal of jowar and the reinclusion of rice, N.M.N. excretion returned to the original low level.

It was calculated that the daily amount of leucine contained in the jowar diet was of the order of 5·3 g. The increase in N.M.N. excretion observed was also of the same order as that obtained with 5 g. L-leucine daily. Supplementation with isoleucine did not influence the N.M.N. excretion pattern on the jowar diet.

Effect of Administration of Large Amounts of Leucine in Pellagrins

In the course of the studies, 5 g. L-leucine daily was administered to 4 patients with pellagra for seven days. This was not associated with any appreciable effects on the clinical picture. The clinical features in 2 other cases in which larger amounts of leucine were given daily were as follows:

Case 1.—The patient was admitted with classical extensive skin changes, oral manifestation of vitamin-B-complex deficiency, and diarrhœa. On admission, apart from a feeling of anxiety, he did not seem to be mentally abnormal. He answered questions promptly and correctly. He continued in this state for four days, during which he was maintained on the basal diet to attain a stable level of N.M.N. excretion. On the fifth day he was given 30 g. of L-leucine in divided doses of 10 g. each at six-hourly intervals. Towards the end of this day the patient was found to behave in an agitated manner, to become incoherent in his talk, and to exhibit delusions of grandeur. The periods of excitement were found to alternate with short periods of severe depression. An additional dose of 10 g. of L-leucine was administered next morning, and by noon the patient became uncontrollable and violent. At this stage leucine was discontinued and he was given nicotinic acid intramuscularly. Within forty-eight hours after the initiation of nicotinic-acid therapy the patient's mental state had reverted to normal.

Case 2.—On admission the patient exhibited the classical skin changes and severe glossitis and had diarrhœa. He appeared alert and answered questions intelligently; he did not show mental changes. He was placed on the basal diet and after the period of stabilisation he was given 20 g. L-leucine daily in divided doses of 10 g. each along with the principal meals. Leucine administration was continued for sixteen days and was then stopped.

Unlike all other cases of pellagra seen by us, in which some clinical improvement results from admission to hospital, even on the basal diet without nicotinic-acid supplementation this patient did not improve clinically; but there was no evidence of worsening of the skin condition or glossitis. Three days after leucine was started, the diarrhœa became more severe and the patient complained of insomnia. On the sixth day the patient became severely depressed and apprehensive; his answers to questions were no longer prompt. When leucine was stopped on the sixteenth day the patient seemed slightly disoriented. Three days after cessation of leucine administration, his mental condition became suddenly worse. He became violent with paranoid delusions and had to be forcibly restrained; his speech was incoherent. At this stage treatment with nicotinic acid was initiated and within forty-eight hours the mental condition returned to normal.

The urinary excretion of N.M.N. was followed throughout the period. Immediately after leucine administration was started there was an increase in N.M.N. excretion, which was maintained throughout the period of leucine supplementation. Three days after leucine was discontinued, when the patient's mental condition had worsened, N.M.N. excretion was still at the same high level as during the period of leucine administration. It was, however, noticed that the increase in the level of N.M.N. excretion brought about by the feeding of 20 g. L-leucine in this case was not significantly greater than the increase observed with 5 g. L-leucine in the healthy subject and other cases of pellagra.

During the period of leucine administration, the patient lost nearly 2·5 kg. body-weight. Within three days after leucine was stopped the weight loss was arrested, and thereafter the patient started gaining weight.

Discussion

While pellagra has long been known to be associated with maize consumption, in the present study the disease has been found to be related to the consumption of the millet "jowar" (*Sorghum vulgare*). The increase in urinary N.M.N. excretion brought about by the replacement of rice by jowar suggests that the greater prevalence of pellagra in jowar-eaters than in rice-eaters may not be

tributable to deficiency in tryptophan or poor availability of its nicotinic acid. It has been shown in the present study that the administration of leucine brings about increased urinary N.M.N. excretion. This finding appears important since both maize and jowar have a high leucine content.

The precise significance of the increase in urinary N.M.N. excretion brought about by leucine requires elucidation. This increase is unlikely to be due to greater availability of nicotinic acid, because both maize and jowar, which are rich in leucine, have been associated with increased prevalence of pellagra. Apparently the aminoacid imbalance caused by relative excess of leucine in diets which are marginal with regard to protein may result in depletion of nicotinic acid from the tissues. The exact role of leucine in nicotinic-acid metabolism needs further investigation.

The increase in N.M.N. excretion brought about by supplementation with 5 g. of L-leucine daily would roughly correspond to the metabolism of an additional 3·5 mg. nicotinic acid daily. In diets which are already marginal with regard to nicotinic acid, this may be an important factor.

It would be premature to draw conclusions from these studies as to the role of leucine in the pathogenesis of pellagra. Case 2, in whom the effects of large doses of leucine was investigated, lost nearly 2·5 kg. body-weight in a fortnight during which leucine was administered. This possibly suggests that the increase in N.M.N. excretion brought about by leucine may not be a specific effect of leucine on nicotinic-acid metabolism, but reflects increased tissue catabolism due to the aminoacid imbalance. On the other hand of 6 subjects—2 healthy and 4 pellagrins—in whom the effect of leucine was investigated, only 2 with pellagra (including case 2) showed reduction in body-weight; in the remainder the increase in urinary N.M.N. excretion was associated with a stationary body-weight.

Summary

Oral administration of 5 g. L-leucine daily brought about an increase in urinary excretion of N-methyl nicotinamide excretion both in healthy subjects and in patients with pellagra. This increase was not influenced by isoleucine.

Isocaloric replacement of rice by jowar brought about an increase in the urinary N-methyl nicotinamide excretion in healthy subjects and in patients with pellagra. This increase also was uninfluenced by isoleucine.

Oral administration of 20–30 g. L-leucine daily to 2 patients with pellagra was associated with temporary deterioration of their mental condition, which was reversed when leucine was discontinued and nicotinic acid was administered.

We are grateful to Messrs. Hoffman La Roche, Basle, Switzerland, for their generous gift of leucine, used in this study; to Mr. S. N. Jagannathan for the blood-nicotinic-acid estimations and Mr. C. M. Manuel for technical assistance; and to the Director of Medical Services, Andhra Pradesh, and the medical officers of the Osmania General Hospital for their cooperation

REFERENCES

Balasubramanian, S. C., Ramachandaran, M., Viswanatha, T., De, S. S. (1952) *Indian J. med. Res.* **40**, 219.

Baumgarten, W., Mather, A. N., Stone, L. (1946) *Cereal Chem.* **23**, 135.

Carpenter, K. J., Kodicek, E. (1950) *Biochem J* **46**, 421.

Rirchjem, C. A. (1956) Some Aspects of Amino Acid Supplementation (edited by E. H. Cole); p. 23. New York.

Kuppuswamy, S., Srinivasan, M., Subrahmanyan, V. (1958) *Spec. Rep. Ser. Indian Coun. med. Res.* **33**, 22.

Mcenney, J. P., Hall, W. L. (1951) *Anal. Chem.* **23**, 983.

Preliminary Communication

SALICYLATE-INDUCED FALL IN PLASMA PROTEIN-BOUND IODINE IN HYPERTHYROIDISM

PREVIOUS work from this laboratory has demonstrated a pattern of metabolic changes produced in normal subjects by a large single dose of sodium salicylate by mouth.[1] This pattern was associated with a fall in plasma protein-bound iodine (P.B.I.) within two hours.[2] A fall in plasma-P.B.I. after long-continued administration of salicylate in therapeutic dosage has also been shown in rats and man.[2] Similar results were reported by Austen et al.[3]

The mechanism of these changes has been discussed elsewhere.[2,4] There is no evidence of a direct effect on the thyroid,[3] although the interference of salicylate with goitre-formation in thiouracil-treated rats indicates that it suppresses production of thyroid-stimulating hormone.[4] Analogy with the fall in P.B.I. after administration of dinitrophenol to rats [5] suggests that salicylate interferes with the normal feedback mechanism—possibly situated in the hypothalamus—which controls the circulating level of thyroid hormone.[6]

We have now studied the effect of salicylate on the raised level of plasma-P.B.I. in hyperthyroid patients.

METHODS

Observations were made on eight patients who had had no treatment beyond rest and sedation in hospital.

The diagnosis of hyperthyroidism was confirmed by the usual investigations—uptake of ^{131}I, basal metabolic rate, and plasma-P.B.I. After a preliminary period of 3–8 days, salicylate was administered orally as calcium acetylsalicylate in divided 4-hourly doses between 10 A.M. and 10 P.M. to a total of 6–8 grammes a day. Fasting blood-samples were taken daily before, during, and after the period of salicylate administration.

Plasma-P.B.I. was determined by a modification of the method of Acland.[7] The finding of Austen et al.[3] that salicylate does not interfere with determination of P.B.I. was confirmed by direct addition of salicylate to plasma and also by addition of salicylate at a level of 70 mg. per 100 ml. to the washed precipitate before ashing. The method described by Trinder [8] was used for the determination of the plasma-salicylate in the same samples.

Measurements of thyroid secretion-rate were carried out after therapeutic doses of ^{131}I in three other hyperthyroid patients, using the technique of Goldsmith et al.[9] The administration of carbimazole 30 mg. 8-hourly prevented the reabsorption of ^{131}I. Serial counts over the thyroid were made with a collimated scintillation detector and the counts were plotted on a semilogarithmic scale (fig. 2).

RESULTS

The effect of salicylate on the first hyperthyroid patient (A) is shown in fig. 1.

From an initial plasma-P.B.I. level of 14·0 μg. per 100 ml. there was a progressive fall to 10·6 μg. by the 4th day of salicylate administration (6 g. per day) when the plasma salicylate-level had reached 41 mg. per 100 ml. Withdrawal of the drug

1. Hetzel, B. S., Charnock, J. S., Lander, H. *Metabolism*, 1959, **8**, 205.
2. Charnock, J. S., Good, B. F., Hetzel, B. S. *Aust. J. exp. Biol. Med. Sci.* 1959, **37**, 473.
3. Austen, F. K., Rubini, M. E., Meroney, W. H., Wolff, J. *J. clin. Invest.* 1958, **37**, 1131.
4. Wolff, J., Austen, F. K. *ibid.* p 1144.
5. Goldberg, R. C., Wolff, J., Greep, R. A. *Endocrinology*, 1957, **38**, 60.
6. Brown-Grant, K. Ciba Colloquia on Endocrinology, 1957, **10**, 97.
7. Acland, J. D. *Biochem. J.* 1957, **66**, 177.
8. Trinder, P. *ibid.* 1954, **57**, 301.
9. Goldsmith, R., Herbert, C., Lutsch, G. *J. clin. Endocrin. Metab.* 1958, **18**, 367.