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Pathogenesis of endemic goitre in Eastern Nigeria

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1. A survey in Eastern Nigeria revealed an area of endemic goitre with a marked variation in incidence from village to village not accounted for by iodine deficiency alone.

2. As dried unfermented cassava was consumed in large quantities in the highly goitrous areas, experiments with rats were undertaken to assess the significance of this factor.

3. Groups of rats were fed (*a*) cassava, (*b*) equal parts cassava and standard diet, (*c*) cassava with added iodine and (*d*) standard diet, and each rat received an intraperitoneal injection of 20 μc iodine 24 h before being killed.

4. The iodine content and hardness of the water in the various areas were estimated and the chemical and bacteriological indices of pollution determined.

5. The following effects were observed: thyroid weight, iodine uptake and plasma-protein-bound iodine were all increased in the cassava-fed animals, the last markedly so. The thyroid's precursor and hormone iodine stores were severely depleted in these animals, which also showed an impaired transfer of iodine from monoiodotyrosine to diiodotyrosine and a high proportion of the iodine present as iodothyronine. The giving of iodine with the cassava did not prevent these changes from taking place.

6. Many of the observed effects suggest that cassava was acting like the thionamide group of an antithyroid drug.

7. These findings are discussed.

In Nigeria, the first attempt to correlate the incidence of endemic goitre with environmental factors such as diet, the quality of drinking water and geological formation was the investigation of Wilson (1954*b*) (see Fig. 1). In a survey carried out amongst schoolchildren, the dispensary patients and communal gatherings in the Niger, Zaria, Kano, Central Plateau and Bornu provinces of Northern Nigeria, Wilson found that the incidence of goitre was fairly high in these provinces (Wilson, 1954*a*). She could not find any relationship between groundnut oil, cereal, salt or the fluorine content of drinking water and the incidence and distribution of endemic goitre. She showed that the distribution of endemic goitre was associated with water samples of low iodine content (0·6–0·7 μg/l.) from pre-Cambrian granites. As stated elsewhere by the author (Ekpechi, 1964), Wilson's excellent work suffers from one serious limitation, namely, that her survey samples could not be regarded as socially and statistically representative of the population of the provinces concerned. The sample was below 0·01% of the entire population of the provinces (Ekpechi, 1964)—well below the minimum of 1% which, according to WHO (1960), is required for a representative sample.

Recently, a more intensive survey carried out by Professor Nwokolo and the author (Nwokolo & Ekpechi, 1966) revealed a high and markedly variable incidence of visible goitre (see Pl. 1) in the Nsukka Division of Eastern Nigeria; this is an area in which the disease had not previously been reported. This area, unlike the areas in which

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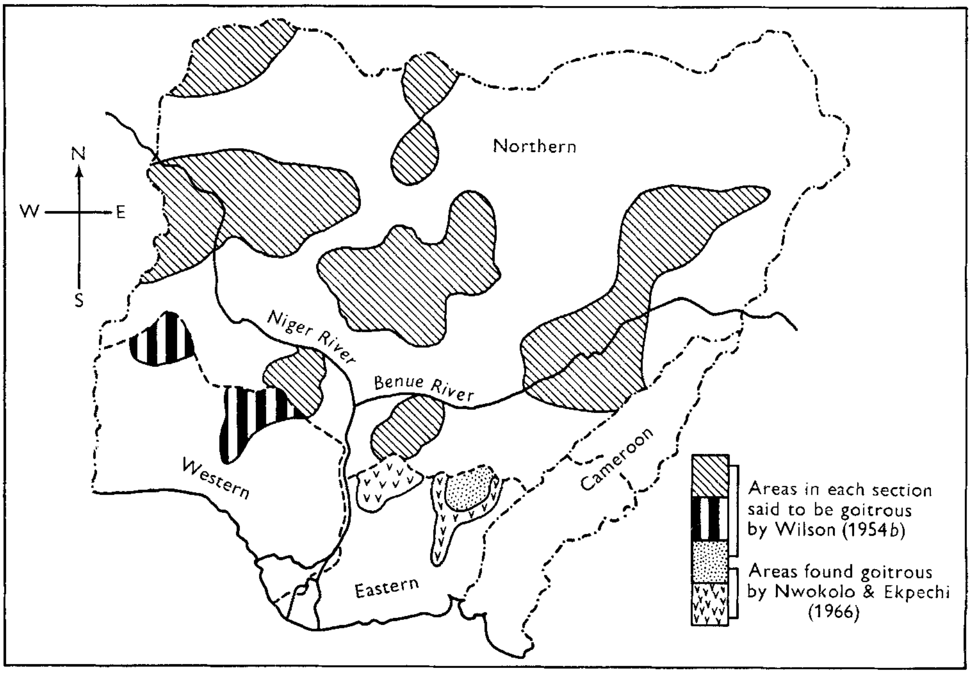


Fig. 1. Map of Nigeria showing the goitrous areas.

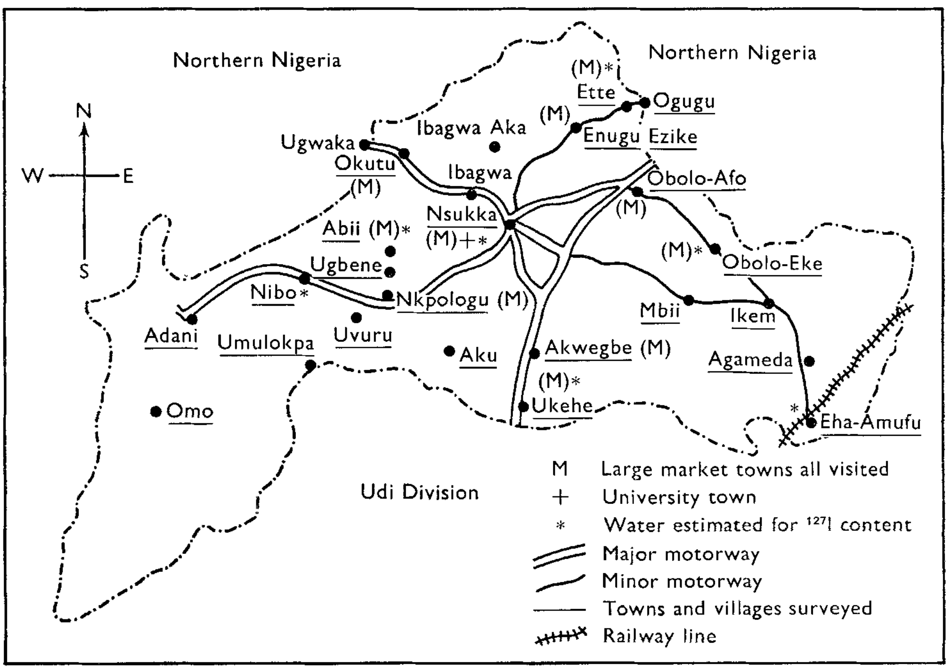


Fig. 2. Map of Nsukka Division in Eastern Nigeria showing roads and principal villages surveyed.

Wilson made her investigations, lies on a much younger cretaceous rock formation (see Table 1 and Fig. 2). A detailed study of the possible environmental factors concerned in aetiology was therefore undertaken. Some chemical and bacteriological analyses of water samples collected from the various villages were carried out. Since the dietary survey showed that much unfermented cassava was eaten in villages with a high incidence of goitre, the effects of cassava meals on thyroid function were investigated in the rat.

EXPERIMENTAL

Chemical analysis of drinking water

Collection of samples. Samples were collected from villages in Eastern Nigeria in accordance with the instructions of the chemist. Sterile 1 l. reagent bottles were used and the samples were collected from the centre of the stream or from water drawn from borehole wells. The samples were taken, within 4 h of collection, to the chemist 40 miles away. The chemical examination of the samples was usually undertaken soon after they were received in the laboratory. The samples for iodine determination were collected in 2 l. bottles, then labelled with ^{131}I and concentrated on a 6 in. column of an ion-exchange resin (De Acidite FF, Cl form, 100–200 mesh Permutit) by being allowed to drip through the resin at the rate of 8–10 drops per min (Ekpechi, 1967). The resin columns were flown back to London for determination of their iodine content.

Iodine determination. The method used was that described by Barker, Humphrey & Soley (1951) and adapted by Dimitriadou, Suwanik & Fraser (1964). The tests were carried out by the author at the Royal Postgraduate Medical School and Hammersmith Hospital, London. As a check on accuracy, London tap water and distilled water were analysed by the same technique.

Other analyses. These were carried out by Mr O. Ekechukwu, Senior Chemist, Ministry of Commerce, Eastern Nigeria, and included determinations of ammonia, chlorides, total hardness as $CaCO_3$, nitrates and nitrites.

Bacteriological examination of drinking water

With samples for bacteriological examination a similar method of collection was used, but the samples were tightly covered soon after collection and despatched immediately. To obtain samples from pumps, the pipe was flamed first and the tap allowed to run for 5 min before samples were taken. The bacteriological analysis for *Escherichia coli* counts was carried out at the Ministry of Health, Enugu, by the Government Laboratory Technologist, Mr Egonu.

Cassava goitrogens

The experiments were carried out on female albino rats weighing 170–198 g. Four groups of three rats each were kept on the following diets, one group on each diet: (*a*) cassava alone; (*b*) 50% cassava + standard laboratory diet, MRC 41 B cubes (Bruce & Parkes, 1956); (*c*) 100% cassava + an iodine supplement of 50 μg ^{127}I/100 ml water, giving 10 μg/rat per day; (*d*) standard laboratory diet, MRC 41B cubes (Bruce & Parkes, 1956).

The object was to study (*a*) the effect of the suspected goitrogen at two different dietary levels, and (*b*) whether the iodine supplement could correct any abnormalities caused by the cassava. The cassava used for the preparation of the rat meals was brought from Nigeria in powdered form. The daily meals were prepared in the Nigerian traditional way by pouring gradually enough powdered cassava into a pot of boiling water, and stirring at the same time until a dark bluish paste was formed. The paste was allowed to cool and then served to the rats. Rats on diet (*a*) and (*c*) were given the cassava meals *ad lib.* and those on diet (*b*) received the same quantity of the paste prepared from equal quantities of powdered cassava and standard laboratory diet (Bruce & Parkes, 1956), mixed in dry form before cooking as described above.

Each rat was injected intraperitoneally with 20 μc carrier-free iodine 24 h before being killed by ether. Immediately after death blood was withdrawn by cardiopuncture and the thyroids were dissected out carefully. The following criteria of thyroid function were measured: (*a*) thyroid weight (absolute and expressed as mg/100 g body-weight, (*b*) thyroid ^{131}I uptake as % dose at 24 h, (*c*) plasma-bound ^{131}I as % dose/ml serum at 24 h, (*d*) thyroid ^{127}I concentration as μg/mg tissue, and (*e*) after digestion and chromatography of the thyroid tissue, the distribution of ^{131}I among the precursors (monoiodotyrosine (MIT) and diiodotyrosine (DIT)) and fully formed hormones (iodothyronines) in the gland. The percentage of the radioactivity in the gland present as iodothyronines, and the ratio of MIT to DIT were used as measures of the efficiency and speed of thyroid hormone production. The methods used have been described previously by Dimitriadou *et al.* (1964).

RESULTS

Examination of drinking water (*see Table* 1)

Iodine. The iodine content of the samples from the Nsukka Division varied from a mean of 0·45 ± 0·01 μg/l. at Abii to 1·35 ± 0·05 μg/l. at Ukehe. Samples from the non-goitrous areas in Eastern Nigeria had an iodine content of 2·4 ± 0·1 μg/l. at Enugu and 6·2 ± 0·07 μg/l. at Onitsha. These values represent the mean of four separate estimations for all the samples analysed. London tap water and distilled water from the Royal Postgraduate Medical School and Hammersmith Hospital, London, on the other hand, showed iodine contents of 17 ± 0·1 μg/l. and 0·06 μg/l. respectively.

Hardness of water. The hardness of all the samples, expressed as $CaCO_3$, was well below 100 ppm which, according to the categories of Jamieson & Parkinson (1958), places all the samples in the range of soft water.

Bacteriological and chemical indices of water pollution. Five of the samples examined had NH_3 content of 0·08 ppm and over, which is regarded by Jamieson & Parkinson (1958) as an index of past pollution. The nitrate content in all the samples was below 5 ppm and the nitrite content was below 1 ppm in all the samples—the latter figure suggesting an absence of recent pollution. Of the eight samples cultured for *E. coli*, only one gave counts high enough to suggest pollution—150/100 ml.

Table 1. *Results of a goitre survey of Eastern Nigeria and some characteristics of the drinking water*

Town or village	Goitre incidence (% with visible goitre)	Chemical analysis of water: Iodine* (μg/l.)	Hardness: $CaCO_3$ (ppm)	Nitrate (ppm)	Nitrite (ppm)	Chloride (ppm)	NH_3 (ppm)	*Escherichia coli* content of water (counts/100 ml)
Nsukka	9·3	0·86 ± 0·09	'Soft'	0·001	1	—	0·021	1
Abii	28·8	0·45 ± 0·01	6	0·001	1	20	2·8	2
Ugbene	27·7	—	4·0	0·001	1	18	0·08	None
Nkpologu	27·8	—	30·0	0·001	1	20	0·08	1
Uvuru	24·6	—	—	—	—	—	—	—
Nibo	28·4	0·58 ± 0·06	18·0	0·001	1	10	0·15	—
Adani	4·0	—	—	—	—	—	—	—
Omo	2·8	—	—	—	—	—	—	—
Aku	2·1	—	—	—	1	20	—	—
Ukehe	3·1	1·35 ± 0·05	5·0	0·001	—	—	0·65	—
Ikem	11·0	—	16·5	0·001	1	10	—	—
Obolo-Eke	25·5	0·61 ± 0·03	8·5	0·001	1	17	1·2	—
Obolo-Afo	12·4	—	28·0	0·001	1	17	0·2	—
Enugu Ezike	1·4	—	12·0	0·006	1·5	18	0·04	1
Ette	32·5	0·84 ± 0·01	3·1	0·001	1	9	0·06	5
Mbii	9·1	—	9·0	0·004	1	8	0·27	—
Akwegbe	1·2	—	32·0	0·002	1	—	0·08	150
Ibagwa	5·6	—	5·0	0·001	1	—	0·04	—
Okutu	25·6	—	—	—	—	—	—	—

* Mean values with their standard errors.

Table 2. *Effect of feeding with cassava on different criteria of the thyroid function of the rat*

(Mean values with their standard errors; three rats/group, given diet for 7 days)

Diet	Thyroid weight		Thyroid ^{131}I uptake	Total ^{127}I	Thyroid digest findings ^{131}I (24 h)		Serum
	mg	mg/100 g rat	(24 h, % dose)	(μg/mg)	Iodothyronines (% gland ^{131}I)	Ratio, MIT:DIT	24 h PB^{131}I (% dose/ml)
100 % cassava	19·1 ± 0·8	11·1 ± 1·1*	18·2 ± 0***	0·14	39·9 ± 1·5***†	1·44 ± 0·06***	0·08
50 % cassava + 50 % standard diet‡	19·3 ± 1·5	10·2 ± 1·4	10·9 ± 0·9*	0·35	33·9 ± 1·0***†	1·4 ± 0·11***	0·02
100 % cassava + iodine supplement (10 μg ^{127}I/day)	16·0 ± 1·3	9·1 ± 1	14·3 ± 0·7	0·36	53·2 ± 2·0***§	1·3 ± 0·02**	0·06
Standard diet (41 B)‡	13·0 ± 0·3	7·3 ± 0·06	14·4 ± 0·7	0·8	25·0 ± 0·8§	0·6 ± 0·02	0·028

MIT, monoiodotyrosine; DIT, diiodotyrosine; PB, plasma-bound.
Differences from controls on standard diet: *, probably significant ($P < 0·05$); **, significant ($P < 0·02$); ***, clearly significant ($P < 0·001$).
† Mostly thyroxine T_4. ‡ MRC 41 B (Bruce & Parkes, 1956). § Mostly triiodothyronine T_4 (see Pl. 2).

Cassava feeding (see Table 2 and Pl. 2)

After feeding with cassava the following changes were observed in the rats. (*a*) Thyroid weight changed significantly. (*b*) Radioactive-iodine uptake by the thyroid increased. (*c*) Plasma-protein-bound radioactive iodine increased markedly. (*d*) The thyroid digest findings showed (1) severe depletion of the gland's precursor and hormone iodine stores, the value for the total thyroid gland being 0·14 μg ^{127}I/mg, (2) an impaired ^{131}I transfer from MIT to DIT, i.e. a high MIT to DIT ratio (1·3–1·44), (3) high transfer of ^{131}I to iodothyronines, a feature also seen in iodine deficiency, but not eliminated when cassava was given with adequate iodine supplements.

DISCUSSION

Evidence of environmental iodine deficiency in the eastern part of Nigeria is provided by the low iodine content (1·5 μg/l.) of randomly selected water samples. Table 1 and Fig. 2 show that the lowest levels of iodine were not found in the villages with the highest incidence of goitre. It thus appears that the difference in iodine content of the samples of water was not great enough to account entirely for the varying incidence of visible goitre in the villages. At Enugu-Ezike and at Ette 8 miles away the incidences of visible goitre recorded were 32·5% and 1·4% respectively. Again, at Nibo the incidence of visible goitre was 28·4% compared to an incidence of 4% at Adani, which is only 12 miles away.

Reports from Columbia (von Humboldt, 1824) and India (McCarrison, 1908), where similar variation in goitre incidence between two adjacent villages has been recorded, show that in Columbia this difference could be accounted for by the varying iodine content of cooking salt used by different villages and in India to the degree of local pollution of water.

All water from the villages proved on analysis to be soft. It is therefore unlikely that hardness of water, as suggested previously by McClelland (1935) in Bengal and by Murray, Ryle, Simpson & Wilson (1948) in England, could be an aetiological factor. None of the water samples showed any evidence of recent pollution, the nitrite content being less than 1 ppm in all the samples. A careful study of the family distribution of endemic goitre failed to reveal any evidence of the influence of abnormal genes in the aetiology of goitre. Unfortunately, it was not possible to analyse the water samples for their fluorine content but it appears that dental fluorosis (mottling of teeth) is unknown amongst the population.

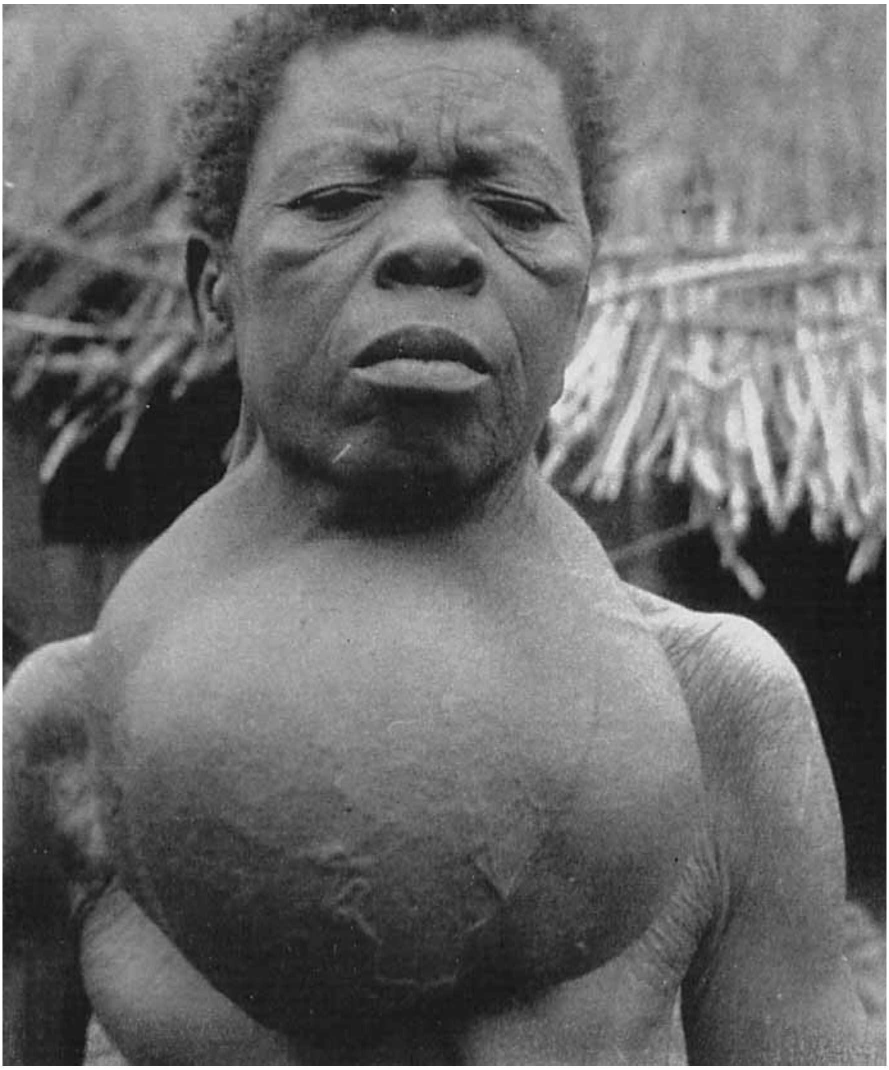
The experimental studies with unfermented dry powdered cassava suggest that the goitrogenic action of unfermented cassava could be attributed either to its low iodine content or to the presence of a goitrogen of the thiouracil group or to both. It is known that cassava has a low iodine content. Cassava feeding, however, led to several changes other than those seen after giving an iodine-deficient diet (Ekpechi, 1964), such as the rapid depletion of the gland's iodine stores and the impairment of the transfer of iodine from MIT to DIT (or a high MIT to DIT ratio). Further, giving the iodine supplement with the cassava meals did not correct these abnormalities. Thus, many of the effects produced by cassava feeding suggest that it contains a goitrogen which acts like the thionamide group of antithyroid drugs, the effects of which are not completely reversed by iodine supplements (see Table 2 and Pl. 2).

Low concentration of thiouracil can cause increased thyroid weight with a variable or even high ^{131}I uptake, severely depleted thyroid iodine stores and a high MIT to DIT ratio (Kilpatrick, 1961; Slingerland & Burrows, 1962). These findings are rather similar to those reported here as occurring after cassava feeding. The high proportion of thyroid ^{131}I found as iodothyronines along with the high serum-protein-bound radioactive iodine are most probably due to low hormone stores in the gland, causing a rapid iodine turnover once the block at MIT: DIT transfer has been passed. Another possible factor might be a diminished peripheral deiodination similar to that which has been reported to occur with thiouracil by Escobar del Rey & Morreale de Escobar (1961).

It would appear that both iodine deficiency and a goitrogen in cassava are possible factors in the aetiology of endemic goitre in the Nsukka Division. Since cassava is a staple food throughout this part of the country, it is likely that it is the added effect of low environmental iodine that determines where goitre occurs. Cassava has long been known (Dunstan & Henry, 1903) to contain a cyanogenetic glycoside. The traditional methods of preparing cassava for consumption involve fermentation which markedly reduces the cyanogenetic glycoside content (Collard & Levi, 1959). It is probable that the goitrogenic property of cassava is closely related to its organic cyanide content. In the villages where dry unfermented cassava is consumed daily during the long dry season, the additional effect of environmental iodine deficiency could probably account for the higher incidence of goitre. This would be in agreement with the observation of Greer & Astwood (1948) that, under conditions of iodine deficiency, the added stimulus of food goitrogens might produce hyperplasia in a thyroid gland which was just able to produce sufficient hormone to satisfy the metabolic requirements of the body while retaining its normal size.

The significance of cassava goitrogens as aetiological factors in this endemic will not be clear until the iodine metabolism of goitrous patients has been elucidated and the effect of cassava meals on thyroid uptake of ^{131}I studied.

I wish to express my gratitude to Professor Russell Fraser of the Royal Postgraduate Medical School, London, and to the late Dr Alice Dimitriadou of the same institution under whose guidance this experimental work was done. I am also grateful to the Wellcome Trust for their financial assistance to complete the work.



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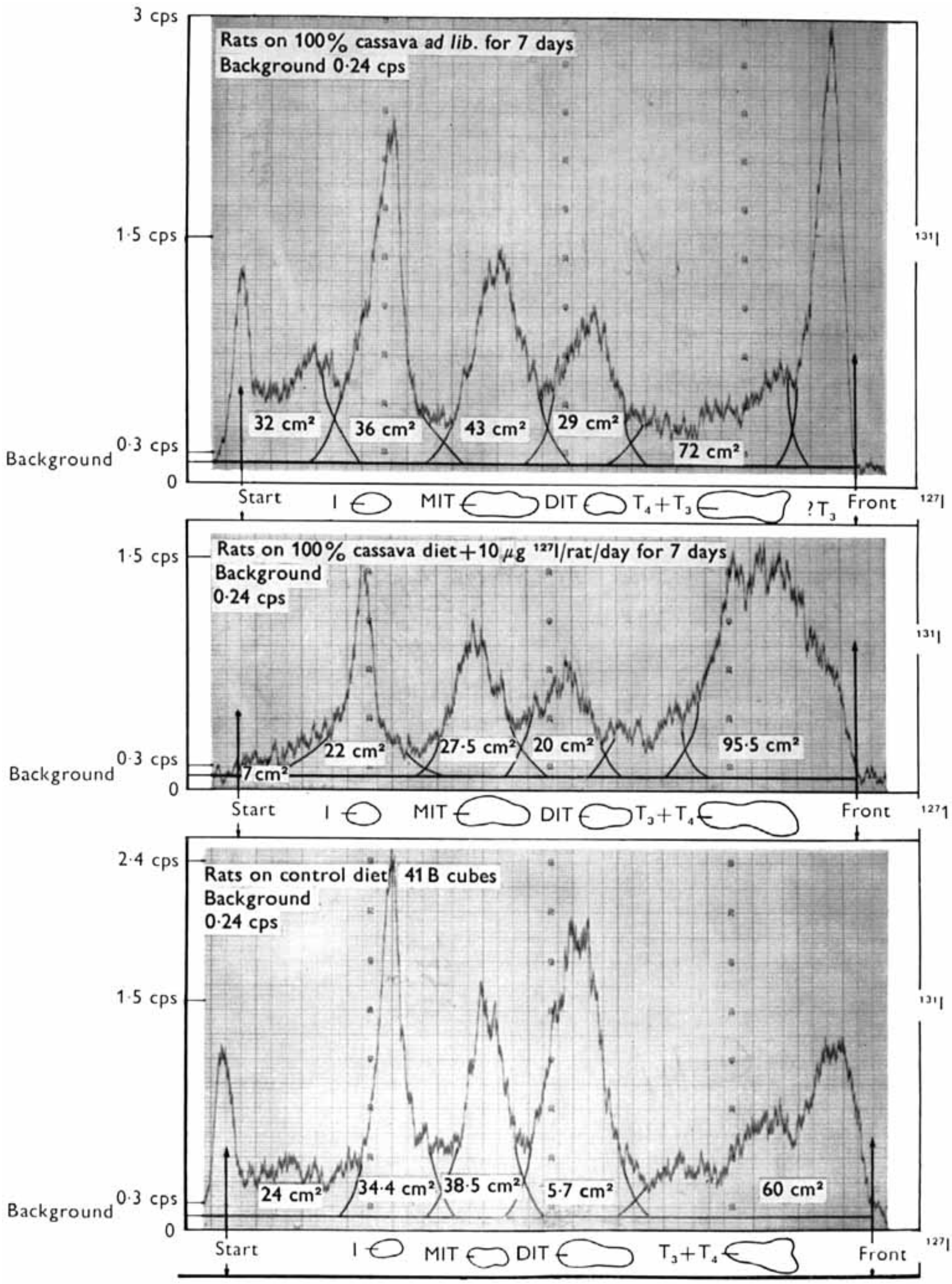

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Rats on 100% cassava *ad lib.* for 7 days
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5·7 cm²
60 cm²
Start
I
MIT
DIT
T₃+T₄
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¹³¹I
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EXPLANATION OF PLATES

Pl. 1. Goitrous patient from Nsukka: an example of obviously visible goitre which is representative of goitre types recorded.

Pl. 2. Chromatogram of resin-purified thyroid digests 7 days after feeding groups of rats on (*a*) cassava meal, (*b*) cassava meal plus iodine supplement, (*c*) control diet MRC 41 B cubes (Bruce & Parkes, 1956). I, iodine peak; MIT, monoiodotyrosine peak; DIT, diiodotyrosine peak; T_3 and T_4, thyronines peak.

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