TREATMENT OF OBESITY BY TOTAL FASTING FOR UP TO 249 DAYS

T. J. THOMSON
M.B. Glasg., F.R.C.P.G., M.R.C.P.
CONSULTANT PHYSICIAN

J. RUNCIE   V. MILLER
M.B. Glasg., M.R.C.P.G.   M.B. Glasg., M.R.C.P.G., D.C.H.
MEDICAL REGISTRAR   MEDICAL REGISTRAR*

STOBHILL GENERAL HOSPITAL AND RUCHILL HOSPITAL,
GLASGOW N.W.

OBESITY is a disease, and its influence on morbidity and even mortality is well recognised. The ætiology is unknown except in a few rare instances, such as Cushing's syndrome, and the treatment consists of eating less food. Anorexigenic drugs are prescribed, but their value is shortlived (Seaton et al. 1961) and they may have the side-effect of stimulating the nervous system; besides, some of these agents pose a serious problem in drug addiction (*British Medical Journal* 1964). Many and varied diets designed to lead to reduction of weight have been advocated. In common with other workers, we have found partial restriction of caloric intake to be relatively ineffective in the treatment of obesity. We therefore decided to adopt the method of total fasting. The metabolic changes during total fasting were first described by Benedict (1915); since then Bloom (1959), Duncan et al. (1962) and Drenick et al. (1964) have treated patients by total withdrawal of food for periods between 10 and 117 days. We describe here our experiences in the treatment of thirteen obese patients by total fasting.

Patients and Methods

The patients were treated in hospital. There were ten females and three males; their ages ranged from 17 to 71 years and their weights from 158 to 286 lb. (71·7–129·7 kg.). A variety of concurrent diseases was present in ten of the patients and in only three was treatment instituted on account of " simple obesity " (see table). All of the patients had failed to lose weight on a variety of weight-reducing regimens. The periods of total starvation in this study ranged from 25 to 249 days. The patients were ambulant in the ward and permitted to drink unlimited amounts of tap-water, acaloric flavoured drinks, and unsweetened tea or coffee without milk.

The volumes of fluid drunk and the urine output were recorded daily. Oral vitamin supplements, were given throughout the period of fasting as two ' Multivite ' tablets, three times a day. Multivite tablets (British Drug Houses) contain vitamin A 2500 units, aneurine hydrochloride 0·5 mg., ascorbic acid 12·5 mg., and calciferol 6·25 µg.

The patients were weighed once weekly and more fre-

* Present address: Hospital for Sick Children, Great Ormond Street, London W.C.1.

quently when special observations were being made (e.g., patient 2). A fresh specimen of urine was examined by ' Acetest ' tablets each morning to detect ketones, and the results were graded. Blood-pressure was recorded daily, in both the recumbent and standing positions. Electrocardiography was performed weekly. Weekly estimations of hæmoglobin, mean corpuscular hæmoglobin concentration (M.C.H.C.), and packed-cell volume (P.C.V.) together with a peripheral-blood film, were made. Serum urea, potassium, chloride, sodium, and proteins (including electrophoresis at intervals), liver-function tests (bilirubin, thymol turbidity, alkaline phosphatase), and serum-cholesterol and uric acid were also estimated. The levels of serum-iron were monitored serially and, in selected patients, a vitamin-C-saturation test was done. The concentration of urea was measured in 24-hour collections of urine in several patients. In four patients, a specimen of jejunal mucosa was obtained by peroral biopsy during the fasting period; in two it was repeated on reintroduction of food. Further investigations were carried out in patients 1 and
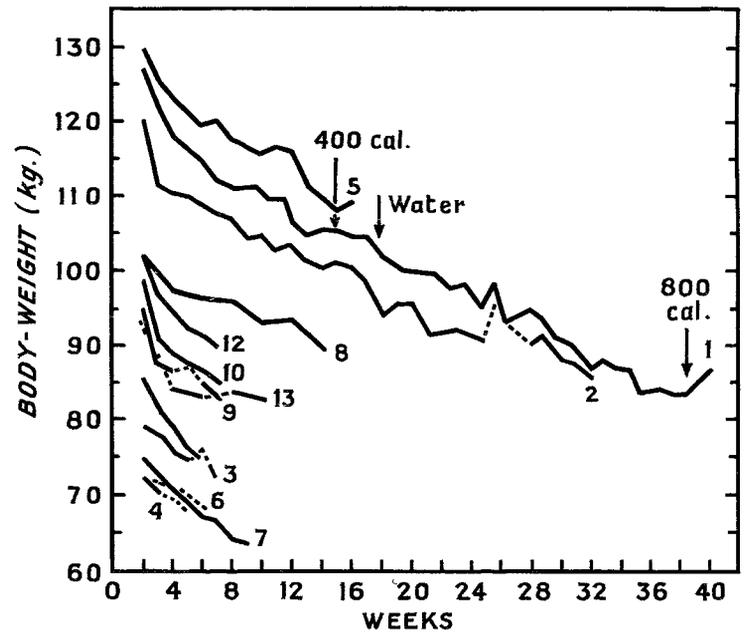

Fig. 1—Weight-loss in 13 patients treated by total-fasting.

2, but these are detailed in the case-reports. During the period of fasting, the mental attitude of each patient was noted. The bowel function and, when relevant, the menstrual history were recorded.

Results

Mental Attitude

Each patient had a strong motive for losing weight, for example, the desire for cosmetic effect in patient 3, incapacitating dyspnœa in patient 2, and determination to be accepted for surgical operation in patient 1. When the details of the therapy were explained to the patients, all were sceptical of their ability to maintain good health

CLINICAL DATA AND EFFECT OF TOTAL FASTING

Patient	Age	Sex	Weight (lb.)*	Diagnosis	Duration of fast (days)	Weight loss (lb.)*	Side-effects
1	28	F	281 (127·5)	Ventral hernia	236	97 (44)	Nil
2	54	F	264 (119·8)	Osteoarthritis, chronic bronchitis	249	75 (34)	Periodic œdema
3	17	F	185 (84·1)	Obesity	39	24 (11)	Nil
4	43	F	158 (71·7)	Ventral hernia	42	9 (4)	Nil
5	34	F	286 (129·7)	Obesity	139	70 (31·8)	Nil
6	61	F	158 (71·7)	Chronic bronchitis	29	8 (3·6)	Nil
7	71	F	165 (74·9)	Hypertension, myocardial ischæmia	50	25 (11·2)	Parotitis
8	61	F	224 (101·6)	Chronic cholecystitis	43	26 (11·6)	Postural hypotension
9	44	F	209 (94·8)	Renal calculus	35	26 (11·6)	Nil
10	48	F	209 (94·8)	Obesity	33	22 (10·2)	Nil
11	53	M	188 (85·3)	Myocardial infarction	27	23 (10·4)	Nil
12	59	M	222 (100·7)	Diabetes mellitus	34	25 (11·2)	Nil
13	52	M	203 (92·1)	Myocardial ischæmia, cervical spondylosis	25	19 (8·6)	Nil

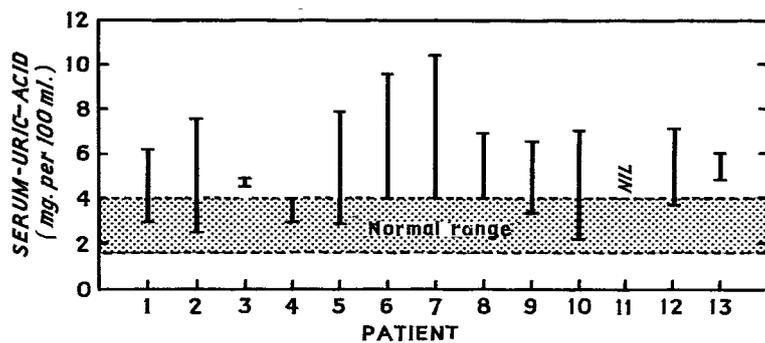* Figures in parentheses show weights in kg.

Fig. 2—Range of levels of serum-uric-acid in obese patients while fasting.

on an acaloric diet. This thought was not expressed again by any patient after the first few days of treatment; confidence was gained by the feeling of wellbeing and the obvious loss of weight.

Weight-loss

All patients lost weight. The greatest loss was 97 lb. (44 kg.) in patient 1, who fasted for 236 days. The graphs for loss of weight are shown in fig. 1. The duration of fasting, the total loss of weight, and the side-effects are noted in the table. Initially the weight fell rapidly, and the loss in the first week was greater the heavier the patient. Patient 2, who weighed 264 lb. (119·8 kg.) lost 17 lb. (7·7 kg.) in the first week. After the first week, the rate of loss of weight usually decreased progressively, but there was individual variation in the rate of fall. Occasionally, temporary weight gain was noted and this was a striking feature in patient 2, where clinical œdema and oliguria coincided with the periods of increase in weight.

Ketonuria

This was noted in every patient, but the semi-quantitative records showed no constant pattern; there was no correlation between the level of ketonuria and the duration of fast or the rate of weight-loss. The patients had no symptoms associated with high levels of ketones in the urine.

Vascular System

The blood-pressure tended to be lower in the fasting state, but the changes were not considered to be significant, with the exception of those in patient 8. In this case, the systolic blood-pressure fell by 20 mm. Hg, when she stood up; this was associated with lightheadedness and, on two occasions, syncope. This was a temporary feature, in that the postural hypotension could not be demonstrated after 5 days. There was no change in pulse-rate in any patient and serial electrocardiograms showed no significant alteration, even in the presence of established ischæmic heart-disease.

Hæmatology

There was no significant change in the level of hæmoglobin, P.C.V., or M.C.H.C. in the thirteen patients after fasting for 1 month. In patients 1 and 2, the serum-iron fell during the later fasting-periods (see case-report). Peripheral-blood-films remained normal as did the total and differential white-blood-cell counts. Hess's test was positive in two patients after fasting for 140 and 180 days respectively; the vitamin-C-saturation test was normal in both of these patients.

Serum-electrolytes remained essentially unchanged throughout the period of starvation. Serum-urea occasionally fell to 10 mg. per 100 ml., but this was not related to the duration of fast; the serum-chloride level tended to be high in some patients (up to 110 mEq. per litre), but

no alteration in the serum-electrolytes could be correlated with the œdema (see patient 2). Serum potassium, sodium, and alkali reserve and plasma-protein were normal in all patients. Blood-glucose remained in the normal range, levels being between 65 and 90 mg. per 100 ml. Serum-cholesterol values were also normal.

Serum-uric-acid

The levels of serum-uric-acid rose in all patients. The range of values in each patient is shown in fig. 2. The highest values tended to be early in the fasting-period, but this was not a consistent finding. There were no manifestations of gout in any patient.

Concomitants of Fasting

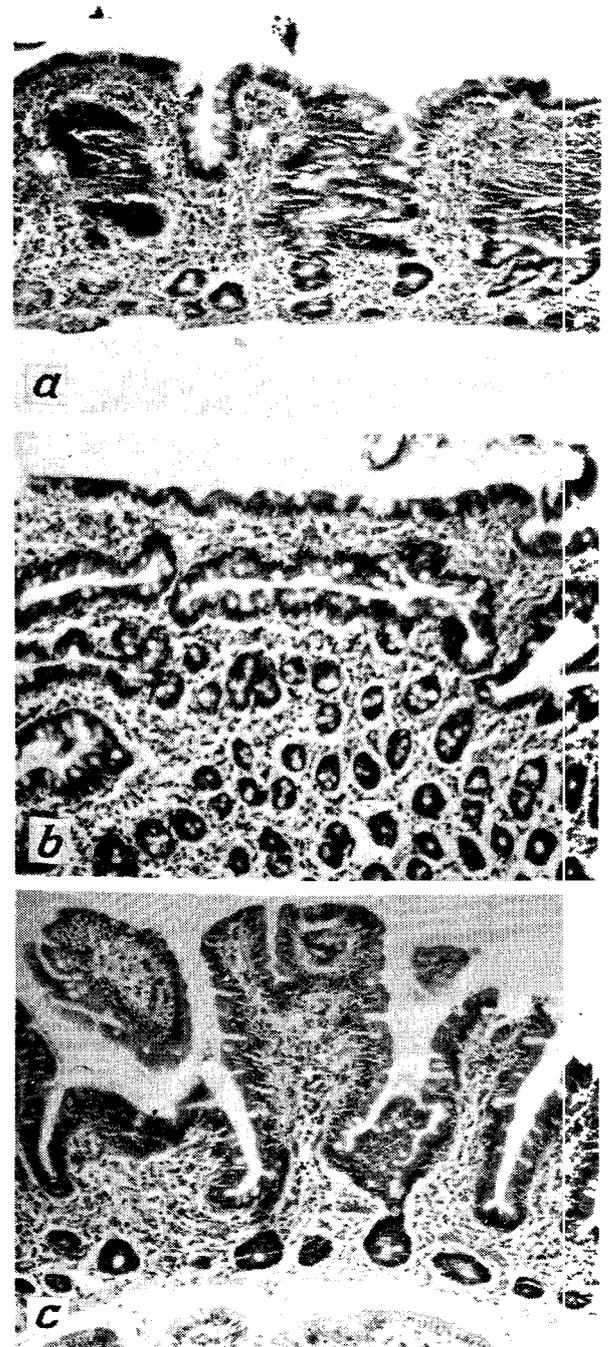Hunger was variable and difficult to assess. We purposely avoided questioning the patients directly, but

Fig. 3—Microscopic appearances of jejunal mucosa.

(a) Patient 1: very stunted leaf-shaped villi.

(b) Patient 12: section on a near-horizontal plane; moderate numbers of inflammatory cells are present.

(c) Patient 2: tongue-shaped and bifurcated villi, shorter than normal.

Hæmatoxylin and eosin; reduced by $^{1}/_{6}$ from × 85.

reports from the nursing staff and spontaneous remarks by the patients made it clear that hunger was a recurrent sensation. It was most notable in the first few days of the fasting-period, but thereafter it mostly subsided, to be stimulated in some patients by the odour of food. There was no detectable physical or mental fatigue, nor were respiratory-rate, body temperature, sleep rhythm, or menstrual function altered.

Biopsy specimens of jejunal mucosa were obtained in four patients (nos. 1, 2, 5, and 12). All were abnormal and showed villous atrophy of varying severity; representative sections are shown in fig. 3. In patients 1 and 12, a further specimen was examined after food had been reintroduced for 13 and 29 days, respectively; these again showed villous atrophy. In all 6 specimens, appearances of the villi under the dissecting microscope were those of leaves and tongues; no finger villi were seen.

Side-effects

The most striking of these was œdema of the legs, hands, and face in patient 2. Other symptoms were vague complaints of headache, lightheadedness, and nervousness, which could not be assigned to any physical abnormality. Since these were mentioned more commonly during the first week of fasting, we considered them to be due to anxiety arising from the absence of food. 1 woman (patient 7) had acute parotitis, which was possibly related to diminished salivary secretion in the fasting-state. The postural hypotension in patient 8 is described above.

Two patients are of special interest; patient 1 who fasted for 236 days and patient 2 who developed severe oliguria and œdema during the fasting-period, which lasted continuously for 249 days. Their case-reports are summarised below.

Case-reports

Patient 1

This 30-year-old woman was admitted to a medical ward for reduction of weight before surgical repair of a large ventral hernia. Her weight was 281 lb. (127·6 kg.) and, except for the obesity and the incisional hernia, physical examination was negative. Blood-pressure was 140/90 mm. Hg. Hæmatological picture, liver-function tests, and level of serum proteins, urea, and electrolytes were all normal. We decided to treat her by total fasting.

After initial hunger, which lasted for two days, she quickly settled down to the acaloric regimen and had no further symptoms which could be attributed directly to the fasting state. After a fast of 90 days, during which she lost 48 lb. (21·8 kg.), we put her on a diet of 400. After 19 days on this diet, her weight had fallen by only 2·4 lb. (1·07 kg.). This reduction in the rate of loss of weight so distressed her that she insisted on returning to the total fast. This was continued for a further 146 days, during which time her weight fell by a further 47 lb. (21·3 kg.). During the total period of 236 days fasting, the menstrual function remained normal.

The following tests were also performed and all values were normal: capillary-blood microastrup readings after 52 and 82 days of fasting; 17 ketosteroids (Medical Research Council 1951), 6·2 mg. per 24 hours; 17-hydroxycorticoids (Smith et al. 1954), 3·9 mg. per 24 hours (at 122 days); serum iron and iron-binding capacity (Ramsay 1957) at 120 days; glucose-tolerance curve (at 180 days); formiminoglutamic-acid-excretion test (Chanarin and Bennet 1962) at 180 days; vitamin-C-saturation test (at 220 days); and bromosulpho-phthalein excretion (at 231 days).

After 236 days of total fasting, she had lost 97·4 lb. (44·3 kg.). During this period, a large number of serial biochemical tests failed to show any abnormality, nor did the patient develop any symptoms which could be attributed to the fasting-state.

Patient 2

A 54-year-old woman complained of severe pain in the right knee, due to osteoarthritis; she also had chronic bronchitis and was dyspnœic on the slightest exertion. She could not do housework. Her weight was 262 lb. (119 kg.). She was admitted to the medical ward for treatment by total fasting.

Hepatic function and serum proteins, cholesterol, and serum-electrolytes remained normal throughout the fasting-period of 249 days. The levels of serum-uric-acid fluctuated, and the range is shown in fig. 2. The standard blood-picture, including peripheral-blood-films, also remained normal. Formimino-glutamic-acid excretion was normal (21 mg.) at 32 days, but was abnormally raised (45 mg.) after 102 days. Serum-iron fell from 90 μg. per 100 ml. to 35 μg. per 100 ml. after 114 days of fasting, the corresponding saturation readings being 28·2% and 15·4%, respectively.

She did not complain of hunger or other symptoms which could be attributed to the fast. The initial weight-loss was satisfactory, 18 lb. (8·2 kg.) in the first 2 weeks. During the 3rd week, however, œdema of the fingers, feet, and periorbital tissues developed, and this greatly distressed the patient; there was an associated increase in dyspnœa. These symptoms subsided after a spontaneous rise in urinary output, only to recur at intervals. The development of œdema was related to changes in the output of urine; there was no alteration in the concentration of urinary potassium. On occasions, the patient's weight would rise by 6 lb. (2·7 kg.) over periods of 3–5 days. At other times, the periods of weight gain were associated with thirst, resulting in an increased intake of fluid, without, however, a corresponding increase in the output of urine. The first such episode was treated by an intravenous injection of 20 mg. frusemide; this produced a satisfactory diuresis, 2790 ml. in the next 24 hours. A further episode was treated by oral spironolactone (' Aldactone A ') in a dose of 50 mg. four times daily. Within 3 hours of the first dose, diuresis began and in the succeeding 24 hours, 3270 ml. of urine were passed. The possible significance of this response is discussed later. The drug was withdrawn on the 2nd day because the patient had a generalised, urticarial rash. The glomerular filtration-rate as measured by the creatinine-clearance test (Owen et al. 1954) was recorded in relation to an episode of œdema. The rate during the œdematous phase was 79 ml. per minute, which is significantly lower than the figures obtained before and after the œdema (117 and 123 ml. per minute, respectively). For the last 73 days of her fast the patient was given frusemide in a dose of 40 mg. daily to combat excessive retention of fluid.

At the end of her 249-day fast, her weight had fallen by 74 lb. (33·7 kg.). She felt greatly improved symptomatically, in that her effort tolerance had increased and she was no longer troubled by her arthritic knee.

Discussion

The principle of the subcaloric diet in the treatment of obesity is well established and the logical extension of this is the acaloric diet. When total fasting was advised as a treatment for patients in this series, the usual response was one of amused incredulity; some patients were frankly apprehensive about its safety and all were sceptical about their ability to continue with such a Spartan regimen. In spite of this, of the first fifteen patients to be so treated, only two did not complete the course, fasting for 11 and 17 days respectively.

The most important factor ensuring success in any weight-reducing regimen is the patient's motivation. The desire to lose weight may stem from cosmetic reasons or from a determination to improve the physical wellbeing. Medical advice and exhortation from relatives are notoriously ineffective in producing sustained reduction of weight by dieting. By contrast, the rapid weight-loss on this regimen presents to the patient, often frustrated and distressed by ineffective dieting, the solution to a hitherto intractable problem. This boost in morale is of inestimable value in ensuring their subsequent cooperation.

The most surprising aspect of this study was the ease with which the prolonged fast was tolerated. Other workers have reported a similar tolerance in their patients (Bloom 1959, Duncan et al. 1962, Drenick et al. 1964); the longest period of fasting reported by these workers was 117 days. We found that intolerance was not a problem, even in patients 1 and 2 after 236 and 249 days respectively.

It is essential to prescribe supplements of vitamins for patients during total fasting. Drenick et al. (1964) found clinical signs of frank vitamin deficiency after 2 months in 1 such patient when vitamin supplements were purposely omitted.

Total fasting is a relatively new method of treatment of obesity. The present study was initially designed to test the feasibility and safety of the method. Drenick et al. (1964) described instances of orthostatic hypotension, normocytic anæmia, and gouty arthritis in patients starved for periods of 12–117 days; Spencer et al. (1966) found no ill effects in three patients who were " starved " (20–30 calories per day) for 12–24 days. Our patients were under close clinical observation and many serial tests of metabolic function were carried out, so that we might correlate side-effects with objective measurements.

The reported liability of ketosis to provoke headache, nausea, and malaise (Kekwick et al. 1959) was not con-firmed. All patients in this series had striking ketonuria, but in no case was this associated with abnormal clinical signs or symptoms. By contrast, all our patients spon-taneously commented on their increased sense of well-being, and in some this amounted to frank euphoria. In fasting patients, Duncan et al. (1963) attributed anorexia to the development of ketosis and considered that return of appetite coincided with its disappearance. Our experi-ence is contrary to this, since the patients did not become anorexic. Cheifetz (1965) has commented on the relation-ship between ketosis and the renal clearance of uric acid; increasing ketosis was associated with diminshing excretion of uric acid and vice versa. Our data does not allow us to make detailed observations on this point, but the highest recorded values of serum-uric-acid in the series were all associated with gross ketonuria and the lower values with periods of lesser ketonuria.

Hyperuricæmia is in invariable accompaniment of the fasting-state and in many cases this is associated with episodes of acute gout (Bloom 1959, Drenick et al. 1964). This high level of uric acid is related to a fall in glomerular-filtration rate (Murphy and Shipman 1964, Cheifetz 1965). Murphy and Shipman (1964) have shown that the renal clearance of uric acid diminishes more rapidly and to a greater extent than does the glomerular filtration-rate. In this series, the expected rise in serum-uric-acid occurred in all cases; but despite values above the normal range (see fig. 2), clinical gout did not develop. This apparent paradox may be related to the known rarity of gout in the West of Scotland; also, the manifestations of gout may only occur as an abnormal tissue response to the elevated levels of serum-uric-acid. It must be anticipated that acute gout could develop during total fasting in other population groups. The precise nature of this renal defect in total fasting is unknown. Since it can be reversed by administration of carbohydrate (Harding et al. 1927), it is tempting to ascribe the abnormality to impaired utilisation of energising substrates, for example, free fatty acids, by the kidney.

The tendency for the level of serum-iron to fall only occurred in prolonged fasting and is a theoretical rather than a practical difficulty. The slow fall is not reflected in the hæmoglobin values.

Important concomitants of the fasting-state are the changes in renal function. Our data at present do not permit detailed analysis of changes in renal activity. The crude tests of renal function—i.e., serum urea and electrolytes—remained normal even on prolonged fasting. The renal handling of uric acid and its inverse relation to the degree of ketosis has already been commented on. In only one of our patients, patient 2, were there more obvious changes in renal function as evidenced by episodic œdema associated with periods of extreme oliguria (urine output falling to less than 200 ml. per day) and consequent rapid weight-gain. This effect is well documented in the fasting individual (Gordon et al. 1963). The cause of these changes is unknown. It is associated with an increased secretion of aldosterone (Rapoport et al. 1965) and increased secretion of antidiuretic hormone (Bansi and Olsen 1959). It has also been related to an exaggerated postural fall in the renal clearance of water in the ambulant, obese patient (Gordon et al. 1963). In this context, the response of this patient to spironolactone during an œdematous phase is worthy of comment. Within 3 hours diuresis began and in the succeeding 24 hours, 3270 ml. of urine were excreted. A further similar episode was treated with bendrofluazide 10 mg. orally, and induced a diuresis of 2400 ml. in 24 hours. This unusually rapid and apparently enhanced response to spironolactone suggests that hyperaldosteronism may be playing some part in this fluid retention. It is note-worthy that there was no associated change in the serum-potassium nor an increased urinary excretion of potassium.

The rather conflicting evidence on the precise mechanisms at play here suggests a third possible effect, that the renal cells of the fasting individual are abnormally sensitive to normal levels of aldosterone and anti-diuretic hormone. We are investigating this by measuring the renal response to intravenous infusions of aldosterone. The results will be described elsewhere.

The abnormalities in the jejunal mucosa are of general interest. They confirm the view of Hindle and Creamer (1965) that such changes are nonspecific and are likely to represent the response of the rapidly dividing small intestinal mucosa to a variety of noxious agencies occurr-ing in widely differing disease states. Similar changes have been found in reticulum-cell sarcoma (Gough et al. 1962), acne rosacea (Watson et al. 1965), and in longstanding diabetes mellitus (Ellenberg and Bookman 1960).

The treatment of obesity by total fasting is the logical extension of the subcaloric diet. We are convinced that it is the treatment of choice, certainly in cases of gross obesity. It is well tolerated by almost all patients. Side-effects are slight and rarely cause subjective upset to the patient, but until further experience has been gained it is essential that treatment by total fasting should only be prescribed under close medical supervision. The results are uniformly successful and frequently dramatic. The fasting-state is associated with changes in metabolism which raise problems of great theoretical interest requiring further investigation.

Summary

Thirteen obese patients were treated by total fasting for 25–249 days in hospital. The investigation was initiated to assess the feasibility of such management. The patients were offered unlimited amounts of acaloric fluids, and vitamin supplements were prescribed.

The fast was well tolerated. Many tests of physiological functions were carried out serially during the fasting period. Side-effects were rare; 1 patient had postural hypotension and another had recurrent œdema. Weight-loss was striking. One patient lost 97 lb. (44 kg.) in 236 days. Total fasting is the most efficient method of reducing weight in obese patients. With our present knowledge, it is recommended that this therapy should only be carried out under close medical supervision.

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EFFECT OF PENICILLAMINE ON HUMAN COLLAGEN AND ITS POSSIBLE APPLICATION TO TREATMENT OF SCLERODERMA

EDWARD D. HARRIS, JR.
M.D. Harvard
CLINICAL ASSOCIATE

ALBERT SJOERDSMA
M.D., Ph.D. Chicago
CHIEF

EXPERIMENTAL THERAPEUTICS BRANCH, NATIONAL HEART INSTITUTE, BETHESDA, MARYLAND 20014, U.S.A.

WE have drawn attention to the feasibility and potential clinical importance of chemical analysis of human dermal collagen obtained by punch biopsy of skin (Harris and Sjoerdsma 1966). We report here the results of chemical analysis in patients who had been treated with D-penicillamine (β,β-dimethylcysteine). Evidence will be presented that penicillamine in doses used for the treatment of Wilson's disease, cystinuria, and rheumatoid arthritis increases the solubility of dermal collagen and decreases intramolecular cross-linking. These effects are similar to those produced in rats by penicillamine (Nimni 1965, Nimni and Bavetta 1965) and by the lathyrogen, β-aminopropionitrile (B.A.P.N.) (Levene and Gross 1959, Martin et al. 1961a). Untreated patients with scleroderma

were found to have less soluble collagen in dermis than controls.

Patients and Methods

Subjects

All subjects were in the age-range nineteen to sixty-five years; 34 served as controls (Harris and Sjoerdsma 1966). The effects of varying durations of treatment with penicillamine were determined by taking biopsy specimens from patients in whom the drug is an accepted form of therapy. Specimens were taken from patients with Wilson's disease by Dr. I. Herbert Scheinberg and Dr. Irmin Sternlieb at Albert Einstein College of Medicine, New York City. Patients with cystinuria were under the care of Dr. Frederic C. Bartter, of the National Heart Institute, and Dr. Stanton Segal, of the National Institute of Arthritis and Metabolic Diseases. Patients with rheumatoid arthritis who were being treated with penicillamine by Dr. Israeli Jaffe, of the Medical College of New York at Flower and Fifth Avenue Hospital (Jaffe 1965), provided a third category of patient for evaluation of prolonged administration of the drug. Except for one patient under Dr. Jaffe's care, patients with scleroderma were in the National Heart Institute. Portions of biopsy specimens from the scleroderma patients were submitted for microscopy to Dr. Leon Sokoloff, who could find no evidence to refute the clinical impression that these patients were in a chronic phase of their disease without manifest cutaneous inflammation or œdema.

Methods

These have been described in brief (Harris and Sjoerdsma 1966). Collagen was measured as hydroxyproline. Purified acid extracts of dermis were lyophilised and stored over calcium chloride at 0°C for up to 1 month. Intramolecular cross-linking in this acid-soluble collagen was estimated by modifications of the method of Reisfeld at al. (1962) and Nagai et al. (1964) for acrylamide disc-gel electrophoretic separation and quantitation of α-(single chain) and β-(double chain) components using densitometry. Plasma-protein hydroxyproline was determined according to the method of LeRoy at al. (1964).

Results

Values for total collagen and α:β chain ratio of soluble collagen in controls and in untreated and penicillamine-treated patients are shown in table I. Ten out of thirteen patients receiving penicillamine had an increase in soluble collagen with an associated increase in the α:β ratio; this increase in the ratio indicates an accumulation of uncross-linked α-chains. The effects of penicillamine were roughly proportional to the dose and duration of therapy. 2 patients (no. 7 and 8, table I), treated with the drug at high levels for five and four years respectively, had levels of soluble collagen more than five times the upper limit of normal, and the α:β ratios indicated pronounced inhibition of cross-linking. 3 penicillamine-treated patients had normal levels of soluble collagen. 1 (no. 9, table I) received the lowest dose (1 g. per day); another (no. 12, table I) took her penicillamine irregularly; and in the third (no. 15, table I) the α:β ratio revealed evidence of the effect of the drug. As shown in fig. 1, the maximum changes in the α:β ratio produced by penicillamine were apparent on simple inspection of the stained disc-gels.

The results in 9 patients with scleroderma are shown in table II. Since biopsy specimens were taken either from the arm and hip, or both, in these patients, similar specimens were taken from 3 controls to rule out the possibility that in healthy adults collagen from the arm and the hip might have different degrees of solubility. 2 of the 8 patients (no. 10, 11, table II) had biopsy specimens taken before and after brief periods of penicillamine treatment. No pre-treatment control specimen was available from patient no. 12 (table II) who had received the drug for nine months. All the samples taken from