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Research Article

PANTOTHENIC ACID DEFICIENCY IN MAN [1]

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For the past seven years we have been studying the role of pantothenic acid in human nutrition and metabolism. This is part of a larger study aimed at defining more clearly the principles of clinical assessment of an essential nutrient in human subjects. It has been assumed that pantothenic acid is necessary for the maintenance of health in man but its abundance in natural foods was such that spontaneous deficiency either did not occur or had not been recognized. Presumably even in very poor diets, other vitamin deficiencies were limiting factors before pantothenic acid deficiency caused definite trouble. At least two avenues of investigation lay before us: to prepare and feed a diet devoid of pantothenic acid, or to give analogs of the vitamin and test possible antivitamin effects. Experiences in devising a deficient diet and testing several antagonists have been described (1–5). Clinical and laboratory abnormalities appeared in healthy young men given an artificial diet, partly synthetic and partly of purified ingredients, the whole virtually devoid of pantothenic acid. The mixture had to be given by stomach tube. The most extensive abnormalities occurred when in addition to the deficient diet large amounts of omega-methyl pantothenic acid were given.

By the time we began the work and subsequently, other investigators described in detail the nature of pantothenic acid deficiency in several laboratory animals (6–10). In growing animals, the earliest evidence of pantothenic acid deficiency is a decline in the rate of growth (11, 12). Strange variations occur in the color of the fur. Exudative lesions appear around the eyes and nose. They are pigmented and contain porphyrin (13). In swine a peculiar neuropathy causes queer stamping and a prancing gait resembling a goose step (14). The fertility of rats is impaired (15) and the fetal death rates of swine rise (16). Sudden death has been noted in several species, often precipitated by hemorrhagic destruction of the adrenal glands (17).

Coenzyme A, the active derivative of pantothenic acid, has functions which are thought to be disturbed and thus cause the metabolic changes observed in animals. Acetylation is retarded, as indicated by a decrease in the portion of para-aminobenzoic acid or sulfonamide excreted in the urine in the acetylated form. Adrenal cortical function is impaired as shown by depletion of sudanophilic and ketosteroid substances from the adrenals of rats, disappearance of the eosinopenic response to adrenocorticotrophic hormone (ACTH), fall in the blood level of glucose accompanied by increased sensitivity to insulin, and defective regeneration of adrenal cholesterol in rats forced to swim for a long time in cold water (18–21).

We designed our tests hoping to detect any abnormalities that might occur at an early and presumably reversible stage. Progress in finding regular abnormalities was slow. Some of the early results were not obtained in later studies. However some clinical and chemical abnormalities were consistent. Fatigue, apathy and malaise characterized the induced illness, with gastrointestinal disturbances common and personality changes and emotional disorders usual. Less regular were signs of cardiovascular instability such as tachycardia and lability of the arterial blood pressure with a tendency to orthostatic hypotension. Several subjects developed paresthesias, burning sensations of the hands and feet, and muscle weakness. In some tests infections were common, in others they were not. Biochemical abnormalities included an inconstant reduction in the degree of acetylation of para-aminobenzoic acid excreted in the urine, a reduction of urinary 17-ketosteroids, a loss of the eosinopenic response to ACTH.

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abnormal glucose tolerance, and increased sensitivity to insulin. Water absorption and elimination were retarded as indicated by the Robinson-Power-Kepler test. Secretion of gastric hydrochloric acid and pepsin was curtailed drastically. Serum cholesterol fell quickly soon after the synthetic diet was started. Hypokalemia developed, accompanied in some instances by electrocardiographic changes. One subject who had many infections had a decrease in gamma globulins but in other subjects they were normal. The results were not completely consistent from test to test, and prompt and complete recovery did not always follow pantothenic acid administration.

We were uncertain whether unrecognized variations in the composition of the diet, the activity of the antagonist, or the inherent variability of the subjects was responsible. Were consistent abnormalities actually manifestations of pantothenic acid deficiency alone or was the diet inadequate in other respects? Did the liquid diet given by stomach tube alter the rate of absorption and utilization of nutrients? Another possible factor was a psychic one. What was the effect of being fed by stomach tube for many weeks? We thought this might alter metabolism as well as mood. Still another possibility was that omega-methyl pantothenic acid might exert some toxic or pharmacologic activity hitherto unrecognized and not related to the function of pantothenic acid. Also, some of the test procedures such as the daily administration of para-aminobenzoic acid or the weekly injection of ACTH might introduce artifacts. Was there an appreciable variability of different lots of omega-methyl pantothenic acid? These were the questions we had in mind in designing the tests herein reported.

THE DESIGN OF THE TEST

We selected six healthy men with the following characteristics:

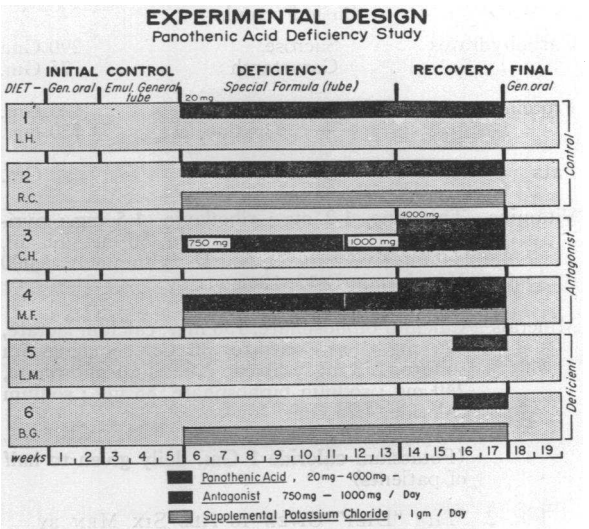

FIG. 1. THE PLAN OF THE STUDY

Each of the larger horizontal bars represents one subject. The diet is indicated at the top and the special regimens are indicated for each pair.

The subjects were paired by weight. During the tests none was aware that his regimen differed in any way from that of the other five subjects. Whenever a pill, capsule or injection was given to one pair or one subject, identical placebos were given to the others. Any procedures done to one was done to all. The general outline of such tests, the material and methods were the same as those in our earlier studies (1–5) except where a difference is noted specifically. Sampling and test procedures were the same for all subjects alike. During the first three weeks of the study, tube feeding of a general hospital diet, emulsified in a blender, was given to detect any psychic or physical effect of this procedure itself (Figure 1). Then we gave a formula very similar to that used in our previous studies (Figure 2). There were differences in the amounts of the various vitamins we gave. We gave 75 per cent of the recommended daily allowances for each vitamin, suspecting that any excesses might mask the effect of a deficiency of pantothenic acid alone. The mineral supplements were changed in one important detail. Previously we had digested the casein with pancreatin in order to get it to go through the gastric tube. The resulting acid mixture

Patient	Age	Height	Weight	Previous health	Physical exam.	B.P.	Group
L. M.	29	73″	180	Cholelithiasis 1954	RUQ abd. scar	125/70	Deficient
B. G.	35	68″	174	"Rheumatism" 1943	Mild obesity	140/75	Deficient
C. H.	26	67″	145	Excellent	Normal	110/70	Antagonist
M. F.	21	70″	135	Excellent	Burn scar rt. abd.	120/70	Antagonist
L. H.	29	71″	170	Excellent	Normal	135/75	Control
R. C.	19	70″	165	Excellent	Dental caries	120/60	Control

	Basic Formula	
Carbohydrates	Sucrose	290 Gm.
	Cornstarch	75 Gm.
Protein	Casein (purified)	125 Gm.
	+ L. Cystine.	750 mg.
Fats	Corn Oil	90 Gm.

Vitamins—Thiamine, 1.2 mg.; riboflavin, 1.5 mg.; pyridoxine, 210 mg.; ascorbic acid, 50 mg.; niacin, 6.0 mg.; vitamin D, 500 U.; vitamin A, 5,000 U.; vitamin B-12, 12 μg.

Minerals—Calcium biphosphate, 136 mg.; calcium lactate, 326 mg.; ferric citrate, 30 Gm.; magnesium sulfate, 138 mg.; dibasic potassium phosphate, 240 mg.; sodium biphosphate, 88 mg.; sodium chloride, 4.25 Gm.

(Potassium chloride 1 Gm. daily given to half of patients)

FIG. 2. THE "DIET" GIVEN TO ALL SIX MEN BY STOMACH TUBE

had a foul odor and was very disagreeable even when taken by stomach tube. We had given part of the sodium as sodium bicarbonate to neutralize the formula. In the present study, a pressure system was devised to force the mixture through the tube. This obviated the need for pancreatin and sodium bicarbonate. The sodium requirement was met by giving sodium chloride.

The experimental design paired the six men in three groups. One pair, L. M. and B. G., received the basic diet devoid of pantothenic acid. Nothing else was given except for the other essential vitamins and minerals, until the end of the "deficient period." At this time pantothenic acid was given in a dose of 4,000 mg. daily.

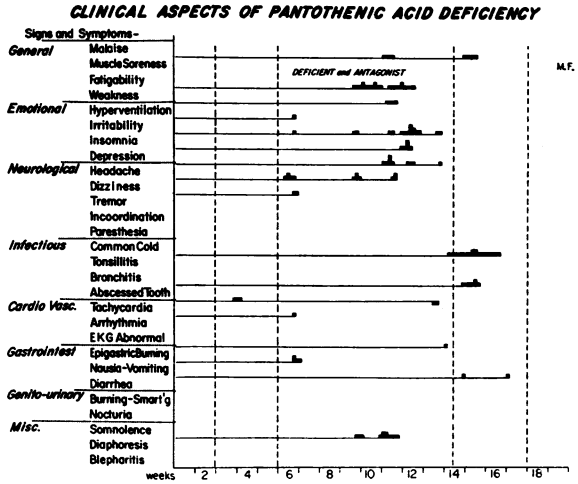

FIG. 3. SYMPTOMS IN M. F. (ANTAGONIST GROUP)

The quantitative aspect of all symptoms is indicated at three levels of mild, moderate and severe by using one, two or three blocks on the vertical axis for each day.

These subjects will be referred to as the "deficient pair."

Another pair, C. H. and M. F., received the same formula devoid of pantothenic acid but, in addition, they got 750 mg. of omega-methyl pantothenic acid. They are referred to hereafter as the "antagonist pair." Later the dose was increased to 1,000 mg. daily because some of the signs and symptoms which they had developed began to diminish spontaneously. At the end of the "deficient period" the antagonist was continued while 4,000 mg. of pantothenic acid daily was added. In the final week of the "recovery period" these two men also received an injection of 3.5 mg. of coenzyme A twice daily.

The remaining pair, L. H. and R. C., served as controls, receiving the basic formula supplemented by 20 mg. of pantothenic acid daily, together with all other essential vitamins and minerals given to the other subjects. These men will be referred to hereafter as the "controls" or the "control pair."

Because of the evidences of hypokalemia encountered in our previous studies, *one man in each pair* got a supplement of 10.8 mEq. of K daily.

Formerly we had found that 10 weeks was the longest period that the subjects could be kept on the experimental regimen of tube feeding. In this study it was possible to tube feed them for 15 weeks, largely because of very close personal care of the subjects, by having better recreational activities, and by the devoted cooperation of the nurses and attendants on the metabolic ward, not to mention the willingness of the subjects to continue a very difficult program.

Methods. The procedures employed to detect biochemical and clinical changes are as follows:

Each subject received his "food" by stomach tube at 11:30 a.m. and at 5:30 p.m. so that special tests and procedures did not delay the two daily meals. Each subject had six cups of black coffee, two bottles of Coca Cola®, and 100 Gm. of hard candy daily. The total intake of calories was 3,200. Each Monday, routine blood counts and urinalyses were done. On Tuesday, blood was obtained for various determinations and a glucose tolerance was done by the fingertip technique (22). On Wednesday, a "Thorn test" was done and 25 units of ACTH was administered (23, 24). On Thursday, gastric analyses were done, (25) and on Friday, an insulin tolerance test followed the withdrawal of blood for routine determinations. On alternate Saturdays a Robinson-Power-Kepler test (26) was performed, and on Sunday, para-aminobenzoic acid was administered in a single 500 mg. oral dose to measure the amount excreted in the urine in the acetylated form (27). Complete urine and stool collections were made daily, using appropriate methods for preservation. Thyroid function studies employed the protein bound iodine (28) and the 24 hour uptake of radioactive iodine (29). Urinary sodium and potassium were determined in a flame photometer but fecal analyses were not done. Seventeen ketosteroids were determined by a modification of the Zimmerman reaction (30). Determinations of nitrogen, cholesterol, total lipids

and phospholipids were done by standard methods (31–33). Serum protein electrophoresis was done by the filter paper method in a Durrum cell apparatus. Samples of the formula were preserved for analysis. A standard graded amount of recreation and exercise was available but the subjects were not required to participate.

RESULTS

Clinical observations

During the three week period of feeding the emulsified general diet, all six men remained in good health except for a few very minor troubles such as a toothache, an occasional headache, an episode of tachycardia and of mild tonsillitis. During the deficient period, as time progressed the antagonist pair developed serious personality

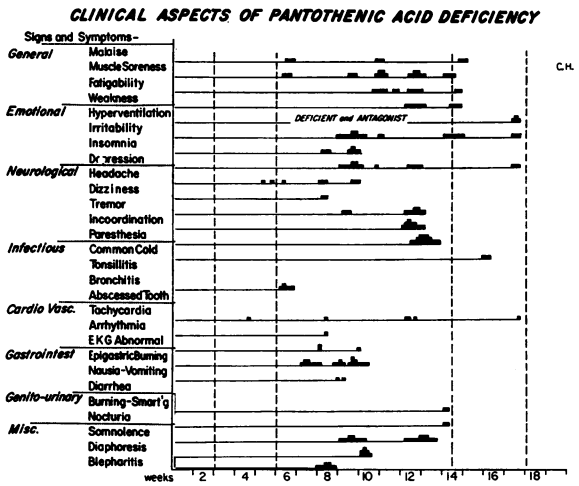

FIG. 4. SYMPTOMS IN C. H. (ANTAGONIST GROUP)

changes with irritability, restlessness, quarrelsomeness; and alternate periods of somnolence and insomia (Figure 3, 4). They began to complain of excessive fatigue after their daily walk. They would break out in a profuse sweat after trifling provocation or none at all. A little later the two men in the deficient group began to note similar complaints (Figure 5, 6). From this time on the condition of the deficient and the antagonist subjects became indistinguishable. All four men had a staggering gait and showed deterioration of their skill at pingpong. Frequently they refused to go for their daily walks, preferring to lie in bed all day. Gastrointestinal complaints became common, varying from epigastric burning to occasional regurgitation of small amounts of formula as they

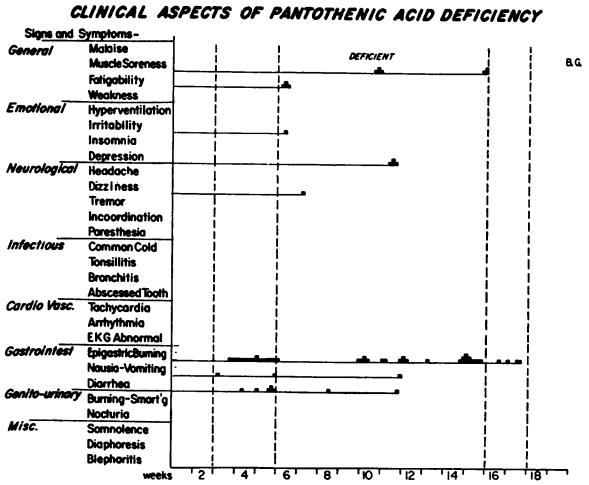

FIG. 5. SYMPTOMS IN B. G. (DEFICIENT GROUP)

withdrew the tube. Loud abdominal rumblings occurred frequently, sometimes accompanied by abdominal cramping and occasionally by diarrhea.

One subject developed paresthesias and "burning" of the soles of his feet which lasted only a few days and subsided spontaneously during the same phase of the test. Numbness of the hands, most distressing in the morning before arising, was fairly frequent in the two subjects receiving the antagonist.

Physical examinations revealed few objective findings other than transient increase of the tendon reflexes and faulty coordination associated with tremor.

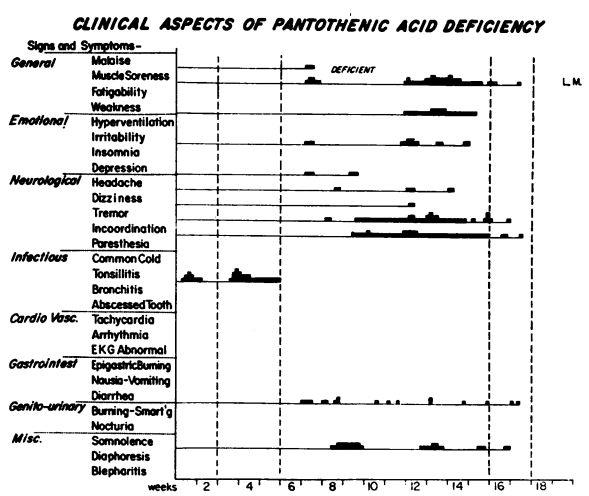

FIG. 6. SYMPTOMS IN L. M. (DEFICIENT GROUP)

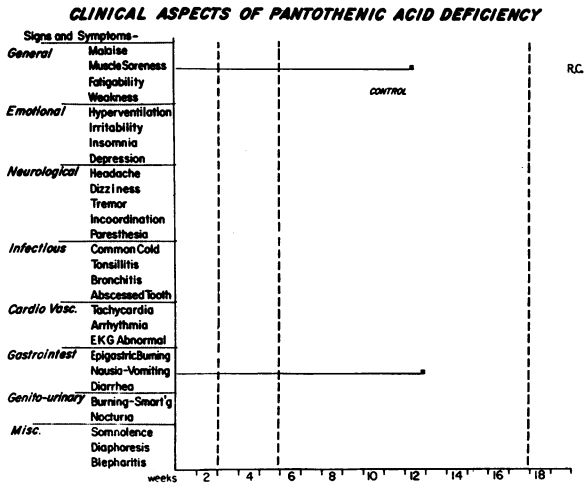

FIG. 7. SYMPTOMS IN R. C. (CONTROL)

The two controls remained well with no more complaints than we have found in any subject constrained in a metabolic ward (Figures 7, 8). The four deficient and antagonist subjects had few cardiovascular symptoms, although one (C. H.) had an arrhythemia which subsided spontaneously before we could get an electrocardiographic record of it. All the men maintained their normal weight throughout the study.

Laboratory results

Routine electrocardiograms remained normal except in one man who developed the T wave changes consistent with hypokalemia. He was getting supplemental potassium chloride and his serum level of potassium was consistently normal.

Blood counts showed a slight decline in hemoglobin, hematocrit and red cell counts resulting from the loss of blood withdrawn for testing. There was no difference in the degree of this mild anemia between the controls and the antagonist or deficient subjects. Urinalysis remained normal.

The erythrocyte sedimentation rate remained normal in the control pair, increased moderately in the deficient pair, but increased more in the antagonist pair (Figure 9). In one of the latter this increased sedimentation rate began to return to normal when large amounts of pantothenic acid were given. In the other, the rate became even faster, returning to normal only after the normal diet was restored.

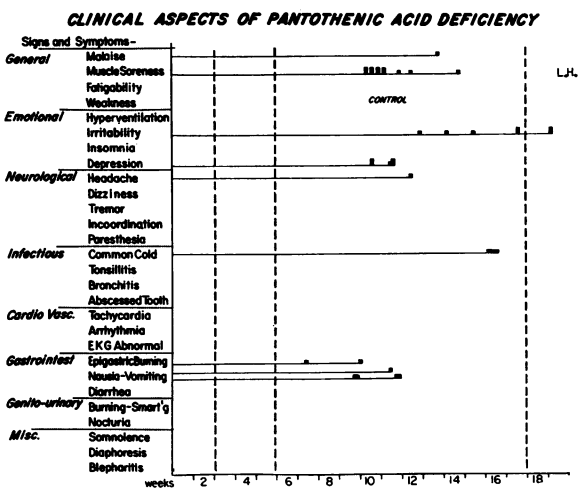

FIG. 8. SYMPTOMS IN L. H. (CONTROL)

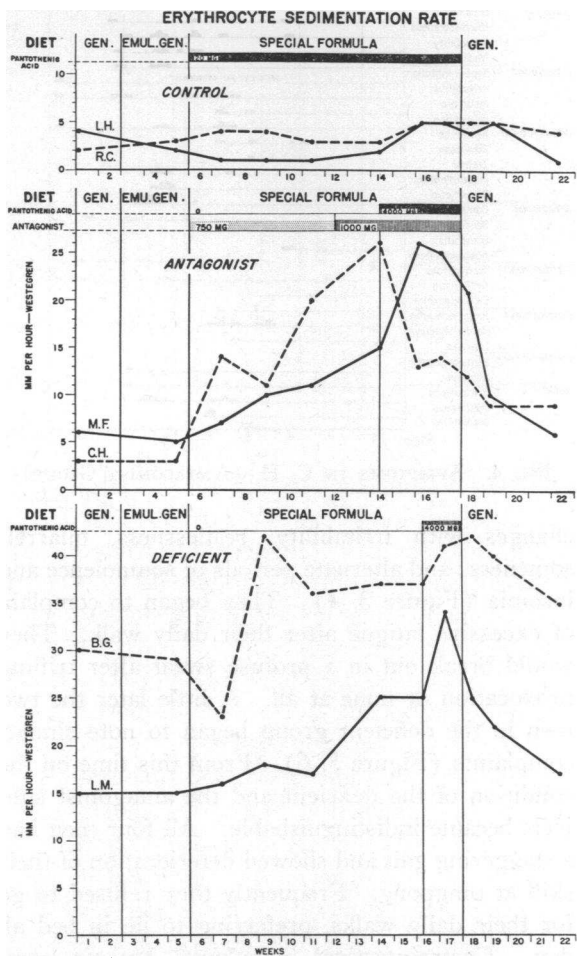

FIG. 9. ERYTHROCYTE SEDIMENTATION RATE IN THE CONTROL, ANTAGONIST AND DEFICIENT GROUP, RESPECTIVELY

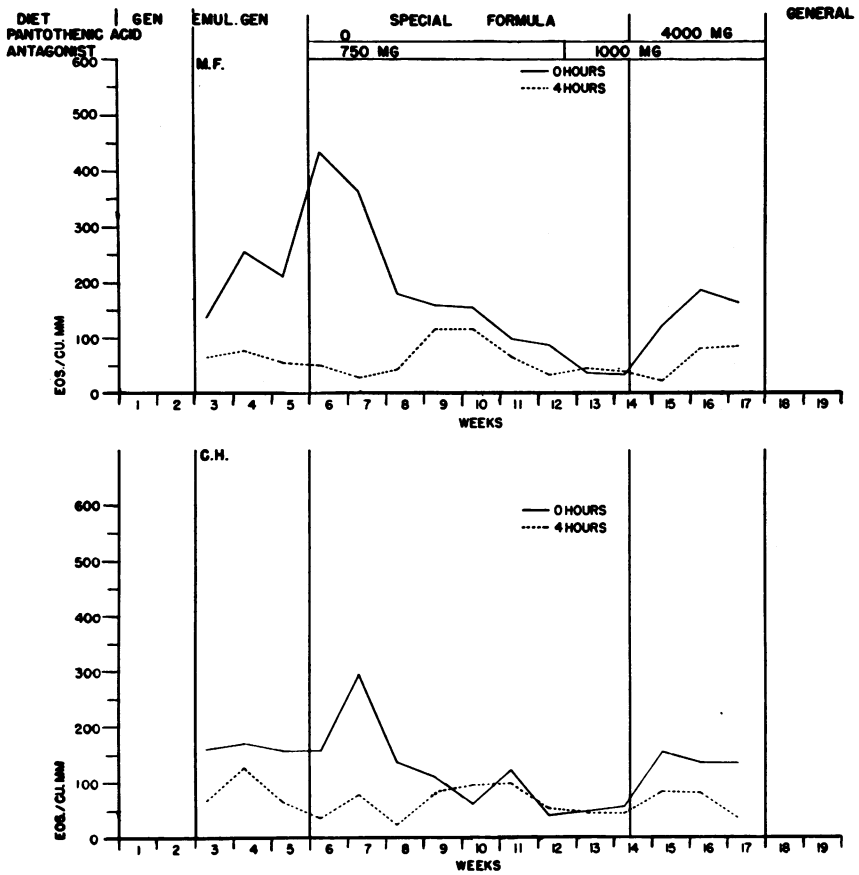

FIG. 10. THE EOSINOPENIC RESPONSE TO 25 MG. ACTH IN THE ANTAGONIST GROUP

The "zero hour" counts are joined by a solid line, while the "four hour" counts are jointed by a dotted line. A normal response is a fall of 50 per cent or more at four hours.

The eosinopenic response to ACTH disappeared in the antagonist pair (Figure 10) and in one of the deficient pair (Figure 11), while remaining normal in the controls (Figure 12). The initial eosinophil counts tended to fall, suggesting that a stress situation existed.

Serum levels of cholesterol and cholesterol esters began to fall soon after corn oil replaced the fats of the general diet. The degree of decline in cholesterol was identical in all three groups. The data are combined in Figure 13. Total lipids and phospholipids did not change during the experiment proper but when the men resumed eating a normal general diet, total lipids increased to twice the control values and there was a slight, consistent rise in phospholipids (Figure 14).

Levels of the serum protein bound iodine and the thyroidal uptake of radioactive iodine rose in all patients despite the fact that all subjects remained euthyroid and none had any clinical suggestion of hyper- or hypothyroidism [2] (Figures 15, 16). These observations will be reported separately.

Liver function, as measured by bromosulphalein clearance tests and serum proteins, remained normal in all subjects. Carbohydrate metabolism was variable, but glucose tolerance curves did not become significantly abnormal. The pattern which we had observed previously in pantothenic acid deficiency—a rapid rise followed by a sharp decline—developed only sporadically in two subjects.

[2] Assay of the formula for iodine revealed approximately 100 mEq. a day. This agrees with the urinary excretion of 86 to 112 mEq. per day by these men.

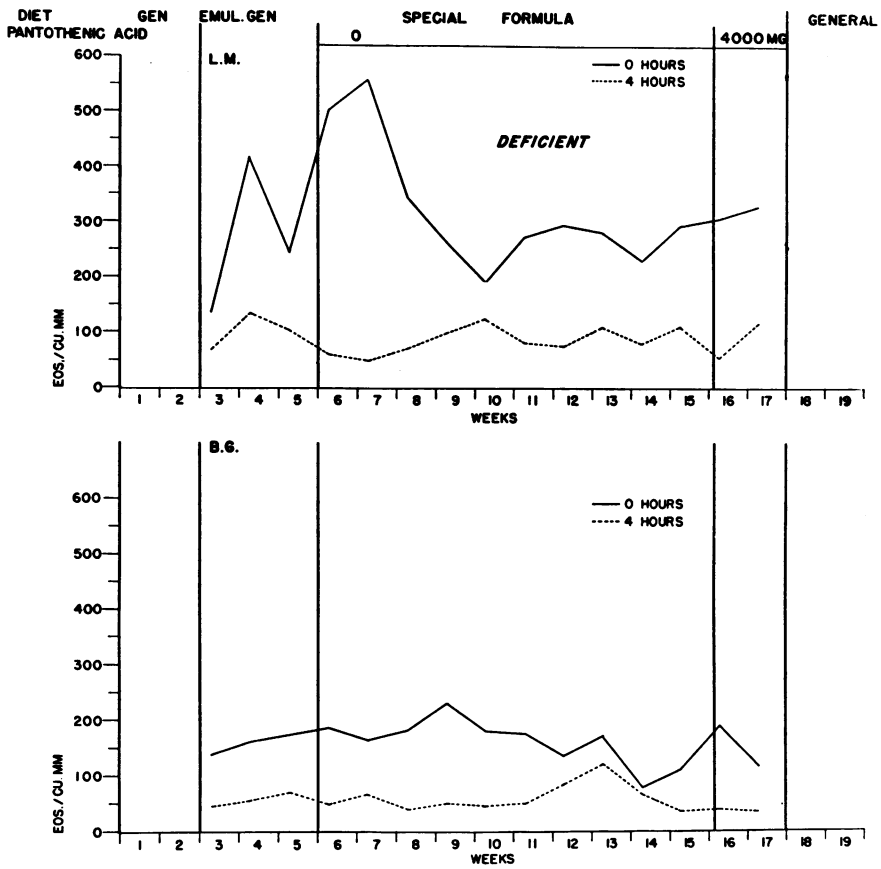

FIG. 11. THE EOSINOPENIC RESPONSE TO 25 MG. ACTH IN THE DEFICIENT GROUP

However, the sensitivity to insulin increased. The usual response to 0.1 units of insulin per Kg. of body weight was a fall of approximately 15 mg. per cent in blood sugar. One antagonist subject (C. H.) developed a 40 mg. per cent decline in blood sugar and complained of symptoms suggesting hypoglycemia (Figure 17). This was corrected by giving pantothenic acid.

The glycogenolytic response to adrenalin was distinctly abnormal in one of the antagonist pair (M. F.). That the drug was effectively absorbed was apparent from his development of tachycardia and slight tremor. In a later test, however, he had a normal rise in blood sugar.

Serum and urinary concentrations of sodium remained normal. For three subjects the formula supplied 20.5 mEq. of potassium daily. This approaches the minimal daily requirement. The other three men received an additional 1 Gm. of KCl daily (10.8 mEq. as K or a total of 31.3 mEq. K). The serum levels of potassium in those without the supplement fell to low normal levels. Those getting the supplement remained slightly higher. Urinary excretion of potassium approximated 10 mEq. per day in the unsupplemented and 20 mEq. per day in the supplemented group (Figure 18). Potassium balance studies were not done. Electrocardiographic tracings did not give evidence of hypokalemia except in the subject mentioned.

Studies of the degree of acetylation of para-aminobenzoic acid (PABA) in the urine failed to show any abnormalities. Similarly the excretion of 17-ketosteroids remained normal. This is in contrast to our previous experiments where the 17-ketosteroid excretion fell markedly.

Studies of the response to water diuresis were too variable to interpret because of a gastro-colic reflex resulting in prompt diarrhea in five subjects.

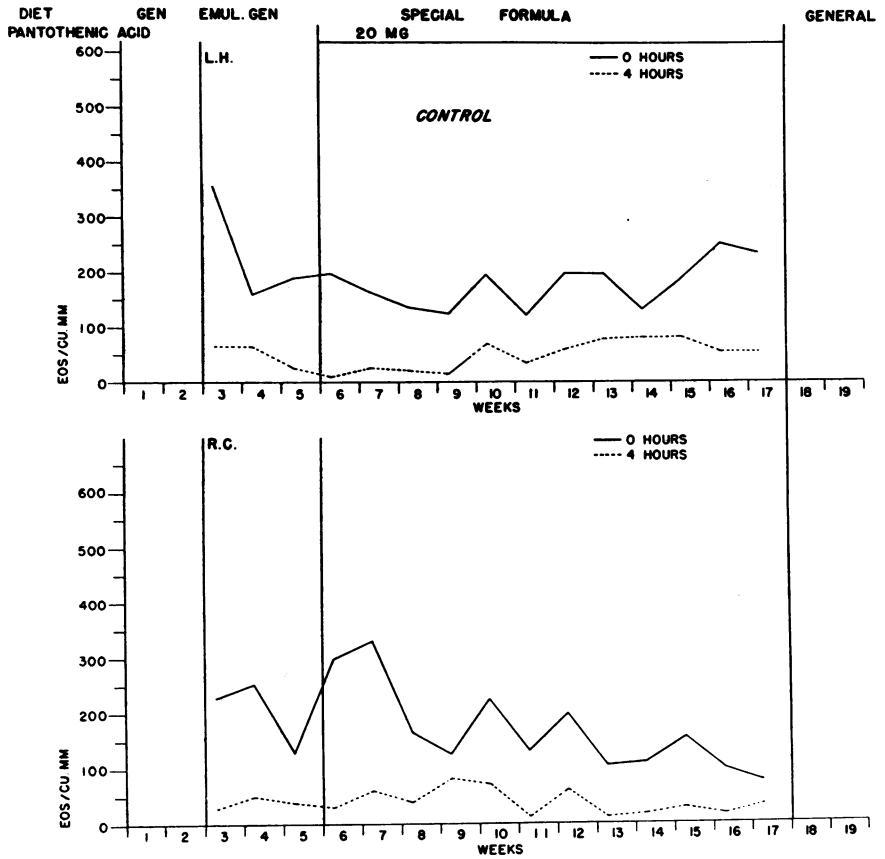

FIG. 12. THE EOSINOPENIC RESPONSE TO 25 MG. ACTH IN THE CONTROL GROUP

Pantothenic acid excretion in the urine was measured by a microbiological technique (34). In the antagonist pair, the results were unsatisfactory because of the inhibition of bacterial growth induced by the urinary excretion of omega-methyl pantothenic acid. While the general diet was being fed to the deficient pair, excretion averaged 4 mg. per day (Figure 19). When the fomula was started, this value declined gradually to less than 1 mg. per day. At the close of this period, when 4,000 mg. of calcium pantothenate was given daily, the urinary excretion rose to approximately 1,000 mg. daily. In the two men who received 20 mg. of pantothenic acid daily, the urinary excretion was about 18 mg. per day (Figure 20).

Nitrogen balance was measured throughout the study in all subjects. There was no striking degree of negative balance in any subject. In the antagonist pair the balance became positive by 2 to 4 Gm. per day after they were given pantothenic acid. When a general diet was resumed this posi-

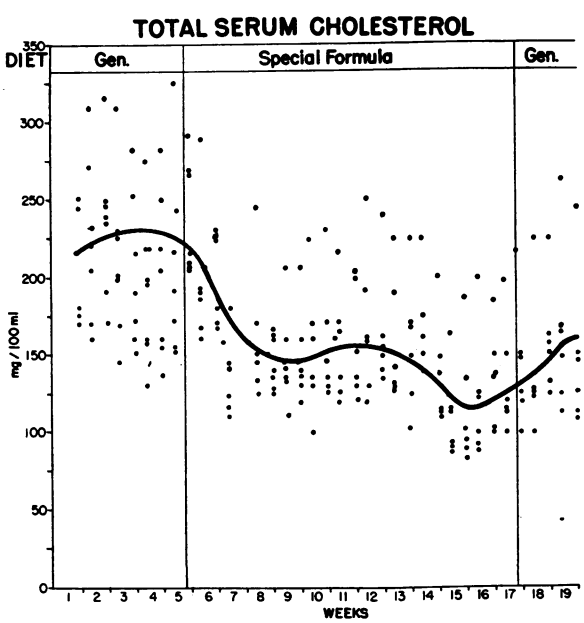

FIG. 13. CHOLESTEROL VALUES OF ALL SIX MEN
There was no difference among the three groups.

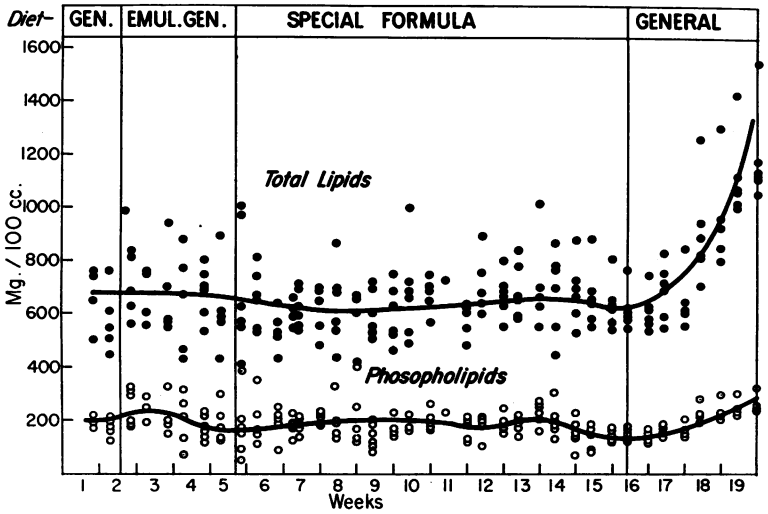

FIG. 14. SERUM FATS

Total fats in all six men remained normal until a general diet was resumed, whereupon the values rose.

tive balance increased further to 7 Gm. per day before decreasing (Figures 21, 22).

Studies of the serum proteins revealed minor and transient decreases of the gamma globulin concentration. The cause for the elevation of the erythrocyte sedimentation rate was not apparent from electrophoretic studies of serum. The gastric secretion decreased as before, but transiently, and to a lesser degree.

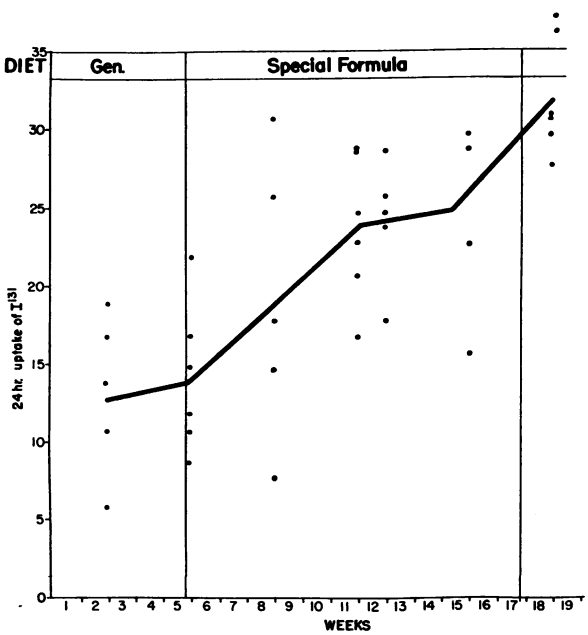

FIG. 15. RADIOACTIVE IODINE UPTAKE

The uptake of radioactive iodine in all six men increased steadily, but did not reach levels found in thyrotoxicosis.

DISCUSSION

This study has clarified some of the aspects of pantothenic acid deficiency induced in man under stringent experimental conditions. With the deficient diet alone, certain changes readily produced by a combination of a deficient diet and the antagonist, omega-methyl pantothenic acid, occurred. The changes were not as early or severe. Omega-methyl pantothenic acid hastened the development and increased the severity of the abnormalities. The effect was quantitative, not qualitative. We have noted that some lots of the antagonist seem to have less antivitamin effect than others. Indeed, one lot seemed to act as pantothenic acid itself. It is possible that the vitamin supplement varied in degree of purity. For these reasons we have found it necessary to employ the bioassay technique to evaluate every substance administered to the subjects. We plan to see whether large doses of omega-methyl pantothenic acid can produce the same abnormal state in subjects eating a normal diet.

The clinical picture of experimental pantothenic acid deficiency includes personality changes, fatigue, malaise, sleep disturbances and such neuro-

logical manifestations as numbness, paresthesias and muscle cramps. Impaired motor coordination also occurs, and may be accompanied by a peculiar gait. Gastrointestinal complaints include nausea, abdominal cramps, occasional vomiting and an increase in the passage of flatus. Epigastric burning sensations are common. Fatigue and headache usually accompany the sensation of weakness; this triad of symptoms is the most constant, persistent and annoying of all. Administration of pantothenic acid was followed by improvement of the paresthesias and muscle weakness, but fatigue and some degree of irritability persisted.

Of the laboratory tests which changed during the deficiency period, the loss of eosinopenic response to ACTH was the most consistent. One possible interpretation of this could be that the "stress" of the deficiency resulted in a maximal degree of eosinopenia (Figure 10) before ACTH was given. If adrenal cortical function had been impaired, other abnormalities should have occurred. Actually only one did occur—an increased sensitivity to insulin. With normal urinary 17-ketosteroids, normal glucose tolerance curves, and normal levels of sodium in blood and urine, we cannot invoke adrenal cortical hypofunction as the explanation.

Potassium requirements in man are taken care of in natural diets. Analysis of the average normal diets reveals that an adult ingests from 50 to 100 mEq. of potassium a day (35). Normally 35 to 90 mEq. are excreted in the urine and 5 to 10 mEq. are eliminated in the feces as unabsorbed or excreted potassium (36). The amounts lost in sweat normally are very small (37). Normal

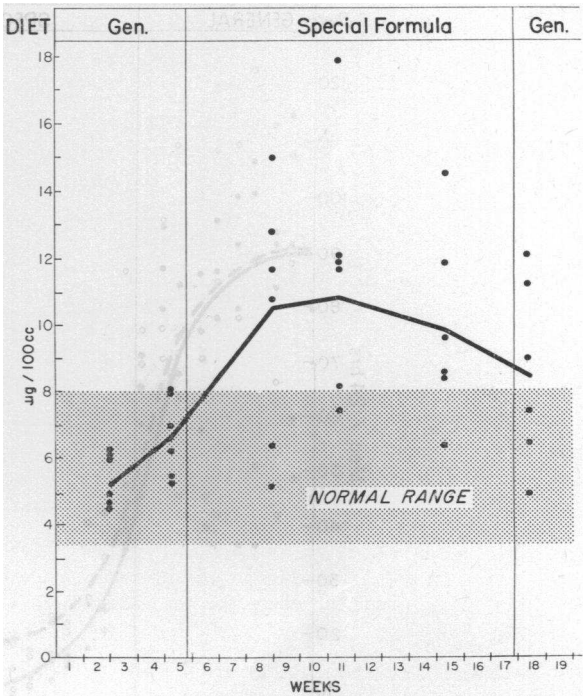

FIG. 16. SERUM LEVEL OF PROTEIN BOUND IODINE
The serum PBI rose to "thyrotoxic" levels in all six men, then began to decline.

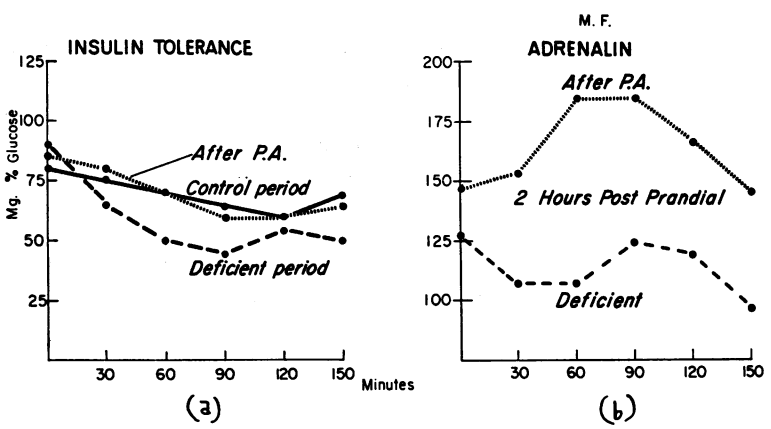

FIG. 17. CARBOHYDRATE METABOLISM
The response to insulin in M. F. (antagonist group) became exaggerated (a). The glycogenolytic response to epinephrine was impaired in M. F. (antagonist group) (b). These abnormalities were corrected by pantothenic acid.

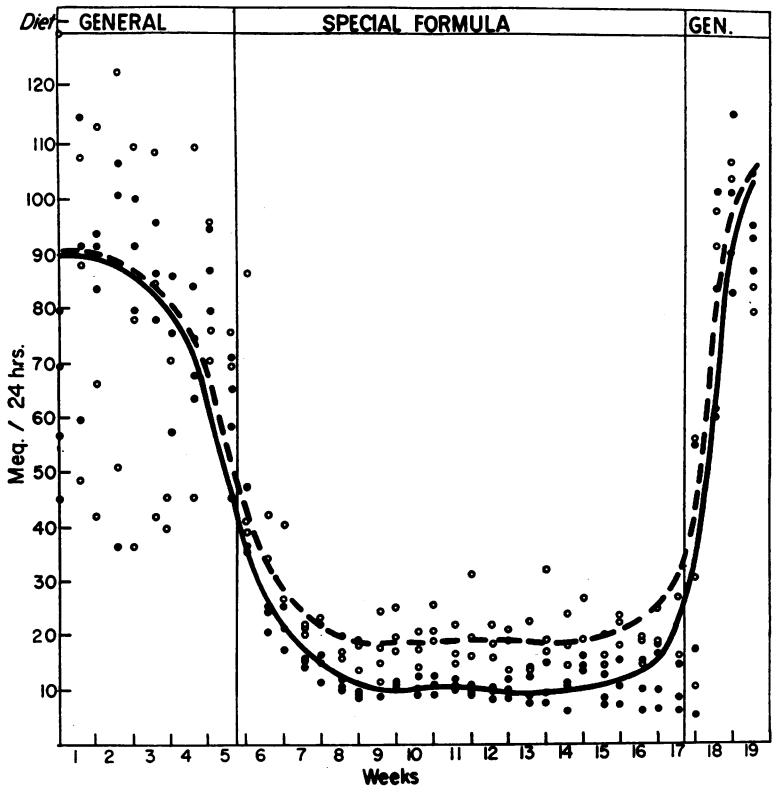

FIG. 18. URINARY EXCRETION OF POTASSIUM

Potassium excretion fell rapidly in all six men and became lower in the men not receiving the extra potassium (solid line). Those with the added 1 Gm. of KCl to the diet had a doubled excretion of K.

kidneys are not nearly so efficient in conserving potassium as sodium (38). Much is known about potassium and other electrolyte depletion in diarrhea, (39) vomiting and prolonged intubation of the gut as well as certain observations in starvation (40).

There were no reported studies of long term potassium requirements in man when we undertook our early investigations. Some of the abnormalities we now know were the result of potassium deficiency and metabolic alkalosis although perhaps pantothenic acid deficiency played a role too. In the present study we made an effort to gather data on the potassium requirement of our subjects by aiming at a minimal level. The urinary excretion of potassium fell rapidly in all subjects when the experimental formula was used. It stabilized at a lower level, ranging from 10 to 20 mEq. a day. On many occasions the serum potassium level of those getting only 20.5 mEq. daily fell to what we accept as the normal lower limit, but they developed no symptoms suggestive of hypokalemia. In the subjects with supplements of potassium the urinary potassium levels were a little higher and blood levels were normal. The slight rise in blood CO_2 did not eventuate in alkalosis.

A transient negative nitrogen balance did occur in C. H. (antagonist group). It was partially reversed during the deficient period and was promptly restored to normal when pantothenic acid was given. There were no substantial changes in the serum protein fractions to explain the increased erythrocyte sedimentation rate. Hypogammaglobulinemia was not a constant finding, although at times gamma globulin levels did decline. The incidence of infections in this experiment was not unusual and was about the same among the three test groups.

The administration of massive doses of panto-

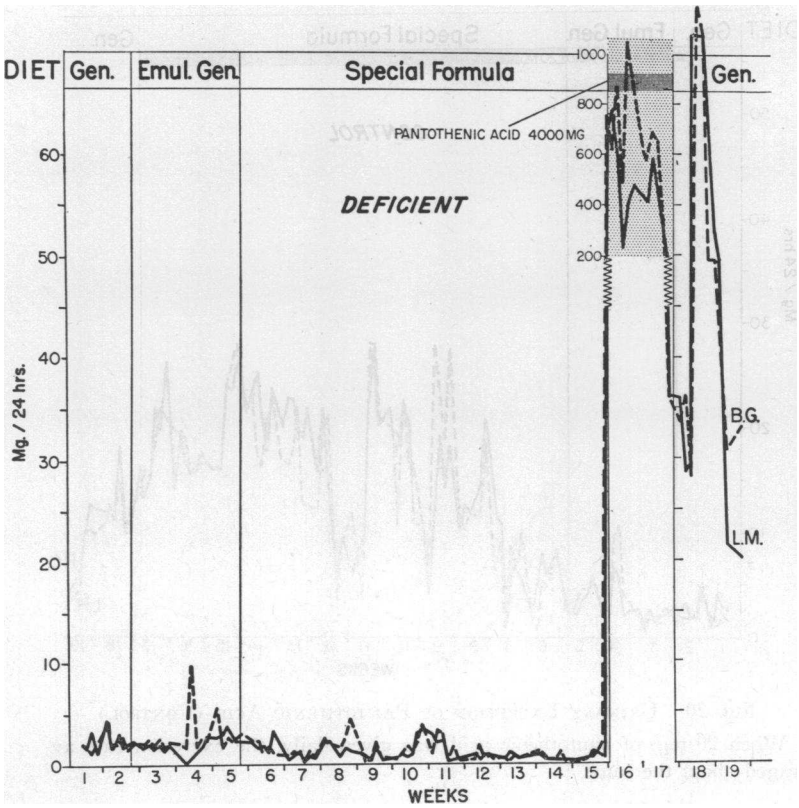

FIG. 19. URINARY EXCRETION OF PANTOTHENIC ACID (DEFICIENT)

Pantothenic acid excretion approached zero after the 11th week of the deficient diet. The men excreted only about ¼th of the 4,000 mg. dose given in the 16th and 17th weeks.

thenic acid to the four men who were deficient was followed by a prompt correction of the faulty eosinopenic response to ACTH and subsidence of most of the clinical symptoms. The erythrocyte sedimentation rate fell significantly in the antagonist pair. There was a coincidental slight rise in the serum cholesterol levels. A most striking response was the positivity of the nitrogen balance. Coenzyme A, which was given to the antagonist pair during the last week, produced no additional effects over those of pantothenic acid.

Especially difficult to interpret are the studies of gastric secretion. In earlier studies, complete histamine-fast achlorhydria developed. In this study, there was a transient fall in gastric secretory activity, followed by spontaneous recovery. It is possible that potassium deficiency or the metabolic alkalosis, or both, were responsible for this disorder of gastric function in earlier experiments. Pantothenic acid deficiency alone probably was not responsible.

Experiments such as these, long as rigidly confining tests, but short in man's life span, impress upon us the wide range of variation even in subjects chosen to conform to a standard. What Williams (41) has emphasized as biochemical individuality and Medawar (42) as the uniqueness of the individual, we see in the personal quirks and inconsistent biochemical patterns which bedevil the framers of human nutritional experiments and make exasperating the necessarily unsatisfactory solution of the problem of controls. We have no doubt that some of the discordant results among our experiments, or within a single test run, come from ignorance and poor planning, but the wide variations in normal persons can easily frustrate

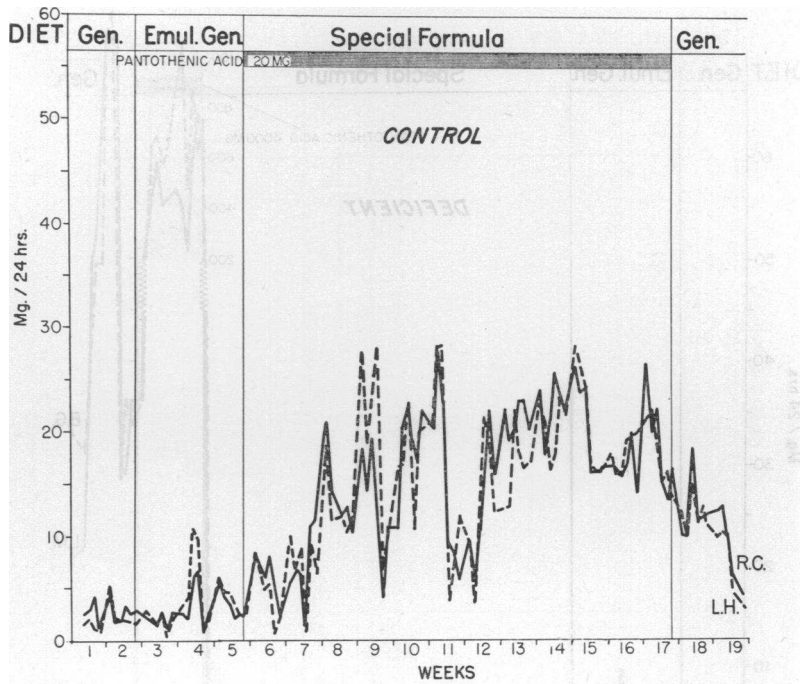

FIG. 20. URINARY EXCRETION OF PANTOTHENIC ACID (CONTROL)
When 20 mg. of pantothenic acid was given daily, the excretion rate averaged about the same.

the best experimental design if the requirement for pantothenic acid should vary manyfold in a small group of subjects.

Perhaps analagous to this individuality, accepted by the clinician but so troublesome to the investigator, is the subtle self-correcting tendency by which homeokinetic adjustments tend to bring the metabolic machinery back on the track after we force it off. We know little of metabolic detours and by-passes which can take over a threatened vital function, though some are recognized. Changes which occur during a period of unchanging experimental procedure seem well enough explained when abnormal signs, symptoms and metabolic function appear to be induced by a dietary deficiency or a deprivation of a specific vitamin. When these aberrations begin to correct themselves by something analagous to the "hunting reaction" of Lewis (43–45) our interpretation must be especially cautious for even the best controls leave much uncontrolled. This self-correcting tendency was a curious feature of some of the signs, symptoms and metabolic functions in our experiments. This emphasizes the need for cautious and critical interpretation of results. Symptoms of distress appeared, only to regress spontaneously with no change in the conditions of the experiment. When we increased the dose of antagonist from 750 to 1,000 mg. per day abnormalities reappeared. Perhaps functions so vital to much of the body's metabolic activity as those mediated by coenzyme A are protected by alternate metabolic pathways when pantothenic acid deficiency is becoming established. We have no data to explain the phenomena, nor clear ideas of how they are brought about.

Review of our errors in interpretation

Some of the abnormalities observed in earlier studies were caused by artifact and inadequacy in experimental design. We mistook these for signs and symptoms of pantothenic acid deficiency. The hypocholesterolemia undoubtedly resulted from the use of corn oil as the source of lipids in the diet (2, 4, 5). The abnormal glucose tolerance curves may have resulted from the rapid feeding of a liquid, partially hydrolyzed diet which could

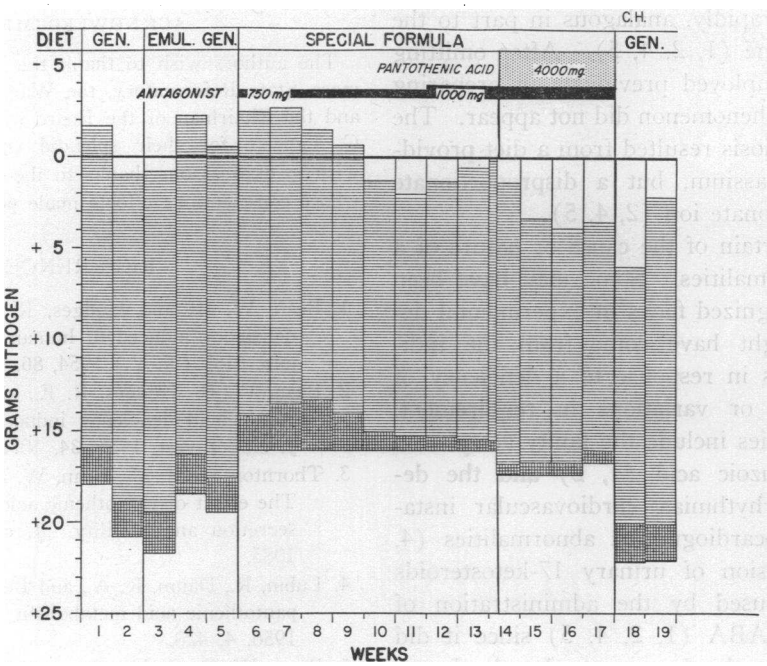

FIG. 21. NITROGEN BALANCE (ANTAGONIST GROUP)

A negative balance is shown by the shaded bar extending above the zero line, a positive balance below it. Administration of pantothenic acid starting the 14th week was followed by a markedly positive balance. This increased when a general diet was fed in the 18th week. The cross hatching indicates fecal nitrogen, the remainder of each bar represents urinary nitrogen, and the base of each bar represents dietary nitrogen.

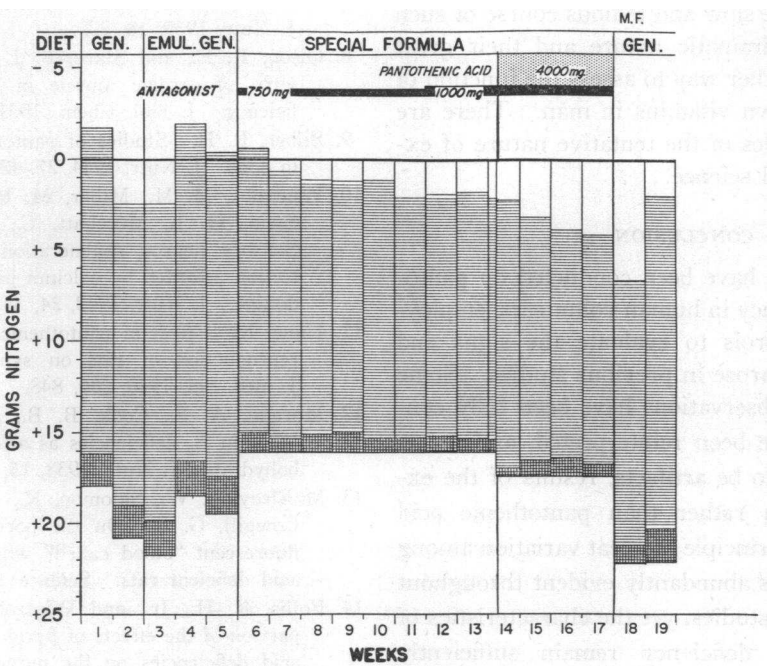

FIG. 22. NITROGEN BALANCE (ANTAGONIST GROUP)

be absorbed too rapidly, analagous in part to the dumping syndrome (1, 2, 4, 5). After omitting the hydrolysis employed previously in preparing the formula this phenomenon did not appear. The hypokalemic alkalosis resulted from a diet providing minimal potassium, but a disproportionate amount of bicarbonate ion (2, 4, 5).

We are not certain of the cause or nature of a few of the abnormalities. Some may have been caused by unrecognized flaws in experimental design. They might have come from the individual differences in response to a deficiency of pantothenic acid or variations in requirement. These abnormalities include the faulty acetylation of para-aminobenzoic acid (1, 2) and the development of arrhythmias, cardiovascular instability and electrocardiographic abnormalities (4, 5). The depression of urinary 17-ketosteroids probably was caused by the administration of large doses of PABA (1, 2, 4, 5) since it did not reappear when the dose was reduced. Large doses of PABA may have an inhibitory effect upon urinary excretion of 17-ketosteroids. The erratic abnormalities of water absorption and excretion in several subjects are not accounted for unless by varying motility.

Even with all precautions we may err in planning studies as well as in interpreting the results. Despite the slow and tedious course of such studies, their undramatic nature and their great cost we have no other way to assess the function of the less well known vitamins in man. There are few better examples of the tentative nature of experimental clinical science.

CONCLUSION

Further studies have been conducted on pantothenic acid deficiency in human volunteers, employing multiple controls to evaluate the signs and symptoms which arose in previous studies. Some of our previous observations have been fully confirmed, some have been reinterpreted, and a few have been found to be artifacts, results of the experimental design rather than pantothenic acid deficiency. The principle of great variation among normal people was abundantly evident throughout this and previous studies, yet the characteristics of pantothenic acid deficiency remain sufficiently clear and consistent to constitute an entity.

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