likely that mucopolysaccharides, which account for approximately 50% of the total connective tissue hexosamine(8) and behave as hydrophilic colloids, would largely account for the parallelism between the tissue water and hexosamine concentrations. The close correlation between hexosamine and water has been observed in experimental myxedema(9), and in human skin(4). This relationship is altered in tissue from fasted rats and in traumatized tissue.

It is well recognized that following many types of body injury the plasma hexosamine level increases(3,5,10). The site of production or source of this hexosamine has not been defined. The synthesis of hexosamine by isolated connective tissue cells has recently been demonstrated(11) and it therefore is possible that the increased amount of plasma hexosamine following trauma may be produced by connective tissue. Although it was not possible to detect changes in hexosamine concentration in non-traumatized connective tissue following trypsin injections(3) or fracture, as reported here, the possibility that a concurrent increase in plasma hexosamine(3,5) may result from its synthesis by connective tissue is not excluded.

Summary. 1. The concentrations of hexosamine and water in connective tissue decrease with growth in the rat. There is a high degree of correlation between the concentrations of water and hexosamine in normal connective tissue. 2. Most traumatic agents (physical and chemical) effect a local non-specific increase in hexosamine and water concentrations. The degree of change of each of these constituents varies with the agent administered. Fasting does not influence this increase in hexosamine in response to local trauma, or the hexosamine concentration in untraumatized connective tissue. 3. Changes in the concentration of connective tissue hexosamine were not detectable following systemic stress (fracture).

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Pantothenic Acid Deficiency Induced in Human Subjects.* (21204)

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Shortly after the classic demonstration by Woods(1) of the molecular antagonism of sulfanilamide by para-amino benzoic acid, one of us (WBB) became interested in seeking similar relationships between individual B-complex vitamins and their chemical analogues. A series of pyridine compounds was studied for vasodilating effects and therapeutic potency against pellagra(2). Observations at that time suggested that some of the compounds might have toxic properties. This suspicion had been expressed by Woolley(3) as a result of his work with canine 'blacktongue'. McIlwain(4) suggested that pyridine-3-sul-

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phonic acid, which antagonized the stimulating effect of nicotinic acid on bacterial growth, might hasten the onset or aggravate the severity of manifestations of pellagra in patients whose poor diets predisposed them to recurrent attacks each spring. Since nicotinic acid, a potent therapeutic agent, was available, an effort was started in 1941 to induce pellagra in patients at the Nutrition Clinic in Birmingham, Ala. In addition to a diet low in B-complex vitamins, these subjects were given injections intravenously of pyridine-3-sulphonic acid for 14 days. Their clinical condition did not change(5). The studies were interrupted for 6 years by the war. However, during these years the first effective use of a vitamin antagonist was reported for the newly discovered folic acid(6,7). Subsequent studies by Vilter and his colleagues(8) demonstrated the feasibility of inducing specific vitamin deficiency states by means of other antagonists. This field of investigation, still in its infancy, has already yielded results in therapy of the leukemias, some neoplastic diseases and in thromboembolic conditions.

The pathfinding studies of Williams(9) in discovering pantothenic acid and of Lipmann (10) in elucidating some of its many functions, have been reviewed elsewhere. Studies of pantothenic acid deficiency in animals have indicated that the principal manifestations are those of adrenal cortical insufficiency, accompanied by low levels of plasma cholesterol, gastrointestinal symptoms, neurological lesions, faulty antibody production and epidermal changes(11-13). Since the role of pantothenic acid in human economy is known only by inference, we undertook a study of this deficiency in healthy young volunteers. With the principle of metabolic antagonism well established, and with injectable pantothenic acid available, we employed antagonists despite some recognized potential hazards (14). Two problems delayed progress. First, we found that most of the foods contained more pantothenic acid than was estimated from the tables, so we finally resorted to a purified and chiefly synthetic diet which was supplemented by known vitamins exclusive of pantothenic acid. This diet alone resulted in some minor biochemical alterations, such as lowering of the plasma cholesterol concentration, and impairment of the eosinopenic response to ACTH, but clinical signs of deficiency did not appear. We next tried the diet plus an antagonist, pantoyltaurine, increasing the dose by small increments until we found that it was inert in amounts many hundreds of times greater than the average daily pantothenic acid intake. The opportunities for testing various clinical and biochemical events seemed almost without limit. We chose those which reflected cortical activity, hepatic function, and acetylation processes. These studies, conducted over 4 years on the Metabolic Ward and supported by generous grants from pharmaceutical manufacturers, various foundations, government agencies, and departmental earnings, set a pattern for our present successful study of human pantothenic acid deficiency.

Methods. We first induced a deficiency in a single subject who was given the deficient diet plus a new antagonist, omega methyl pantothenic acid. From this pilot study we formulated an experimental design which was applied to 3 additional subjects simultaneously. The subjects were 4 healthy men ranging in age from 19 to 31 years. One had bronchial asthma, but was otherwise well. The other 3 were without illness. The plan was to make baseline studies for a 12-day period. During this time the men were to receive a 'basic diet' fully supplemented by all nutritional requirements. The second period was to be identical except for elimination of pantothenic acid from the diet, and substitution of 0.5 g of omega methyl pantothenic acid daily. Its duration was to depend upon clinical or biochemical evidences of a deficiency. Finally, we planned a third period, identical with the second, but adding large amounts (4 g) of pantothenic acid to overbalance the effects of the antagonist. We proceeded according to this schedule, through the first period, and the second which was ended when certain abnormalities developed. However early in the third period, when it became evident that the clinical manifestations of the deficiency were progressing despite the addition of pantothenic acid, we promptly abandoned the proposed schedule and instituted emergency therapy

TABLE I. Clinical and Laboratory Changes in 3 Men Deficient in Pantothenic Acid.

	I	II	III	IIIa	IIIb
	Control 12 days	Deficiency 35 days	Replacement 6 days	Emergency therapy 10 days	Recovery 14 days
	Basic diet Vitamins Minerals	Basic diet Vit. (–P.A.) Minerals Antagonist	Basic diet Vit. (+P.A.) Minerals Antagonist	Cortisone (3d.) General diet Parenteral B-vit. and P.A.	Basic diet Vitamins Minerals
Clinical observations					
Wt	N	N	N	++ (edema)	N
Blood pressure, syst./diast.	N/N	±/—	±/—	+/+	N/N
Skin & mucous membranes	N	N	N	N	N
Personality	N	—	—	+	N
Gastro-intestinal changes	N	++	++	+	N
Appetite	N	—	—	+	N
Neurologic stepp. gait	0	+	+	0	0
paresthesias	0	+++	0	0	0
tendon reflexes	N	++	++	N	N
vertigo	0	++	+++	0	0
burning feet	0	+	+	0	0
Sense of well being	N	—	—	±	N
Laboratory tests					
17 ketosteroid	N	—	—	—	N
Eosinopenia—ACTH	+	—	±	+	+
Cholesterol and esters	N	—	—	—	—
Blood counts—RBC	N	N	N	N	N
Hb	N	N	N	N	N
Wbc	N	N	N	N	N
Eos	N	+	+	N	N
Diff.	N	N	N	N	N
Urinalysis—Sugar	0	±	±	±	0
Albumin	0	0	Tr	Tr	0
Micro	N	N	N	N	N
Urobilinogen	Tr	0-+++	0-+++	0-+++	Tr
Glucose tolerance	N	±	±	±	N
Insulin "	N	±	—	±	N
Water diuresis	N	—	—	±	N
Gastric acidity	N	—	—	±	N
Plasma proteins	N	N	N	N	N
BSP, 5 mg/kg/45 min.	N	N	N	N	N

Key: N = Normal, + = Increase, — = Decrease, 0 = Absent, Tr = Trace.

until recovery was clinically evident. At this time the third period was resumed, and observations again obtained. Among the measurements selected to detect early signs of deficiency, we included daily recordings of body weight, blood pressure, urine volume, acetylation of administered PABA, and excretion of 17 ketosteroids. Twice each week, we determined plasma cholesterol and cholesterol esters, eosinophil response to ACTH, and gastric acidity. Once a week, we obtained a complete blood count and urinalysis. The glucose tolerance, insulin tolerance, and response to water diuresis were also observed weekly. Each 14 days we took a photograph of the men and determined plasma proteins, plasma electrophoretic patterns and bromsulfalein extractions.

Results. Clinically, no changes were evident during the first period. But in the second week of the period of deficiency we noted a fall in the diastolic and lability of systolic blood pressure. Postural hypotension and vertigo developed, accompanied by tachycardia after slight exertion. The men complained of easy fatiguability and slept frequently during the daytime. By the third week of the deficient period, they complained of occasional bouts of epigastric distress accompanied by anorexia and constipation. One subject regurgitated occasionally, necessitating caloric replacements. The fourth week found

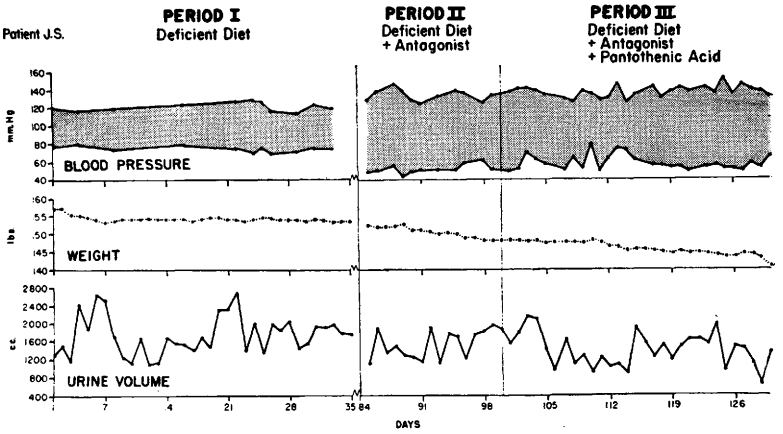

FIG. 1. Pt. J.S. Note development of wide pulse pressure accompanied by progressive wt loss despite dietary intake of 3000 calories with 100 g of protein daily. Addition of pantothenic acid did not promptly reverse these changes.

the subjects discontented, quarrelsome, irascible, and easily upset. They complained of increasing sensations of numbness and tingling of the hands and feet. In the following week, these sensations became more annoying, and in one man consisted of a constant burning of the feet. Another developed a steppage gait, while the third complained of constant paresthesias. Neurological examination disclosed hyperactive deep tendon reflexes, inability to walk on tip-toe, weakness of the extensor muscles of the fingers, and impaired sense of balance. Nevertheless, objective sensory examinations were normal as were the plantar reflexes. During this entire period of deficiency, the men had frequent upper respiratory infections, especially acute pharyngitis, which previously had been infrequent. One developed pneumonia which responded rapidly to antibiotic therapy.

At this time, it was deemed necessary to restore pantothenic acid to their diets, so the third period was started. Although the paresthesias improved promptly, and the eosinopenic response to ACTH was partially restored, the patients became more fatigued, and their sense of well-being deteriorated. At the same time, their urinary excretion of 17 ketosteroids became lower. Severe vomiting suddenly developed in one patient, while another became somnolent and lethargic for an entire day, although he had no further alterations in his pulse, respirations, blood pressure or temperature. These alarming events, reminiscent of acute adrenal insufficiency, led us to institute prompt therapy with fluids intravenously and cortisone parenterally in the first patient, and cortisone orally in the other 2. They were also given a general diet supplemented by multiple vitamins and injections of B-complex vitamins and pantothenic acid. In response to this therapy, the men improved rapidly and cortisone was discontinued after 3 days. The diet and vitamins were continued for another 7 days during which time the men gained weight rapidly (10 to 13 lb), and developed edema and elevated blood pressure. At the same time, their urinary excretion of 17 ketosteroids declined still further. The next 2 weeks, during which time the "basic diet" supplemented by vitamins was resumed, were characterized by gradual recovery both clinically and biochemically. No further evidence of neuropathies occurred, but one man who had had thrombophlebitis 8 years previously, had a mild recurrence with residual edema of the affected foot and leg.

The general features of biochemical changes accompanying pantothenic acid deficiency, presented in Table I, are characterized by impaired ability to acetylate PABA, and a decline in blood levels of cholesterol and cholesterol esters. Some evidences of adrenal cortical hypofunction are found in the eosinophil response to ACTH, the increased sensitivity to insulin, the decreased urinary excretion

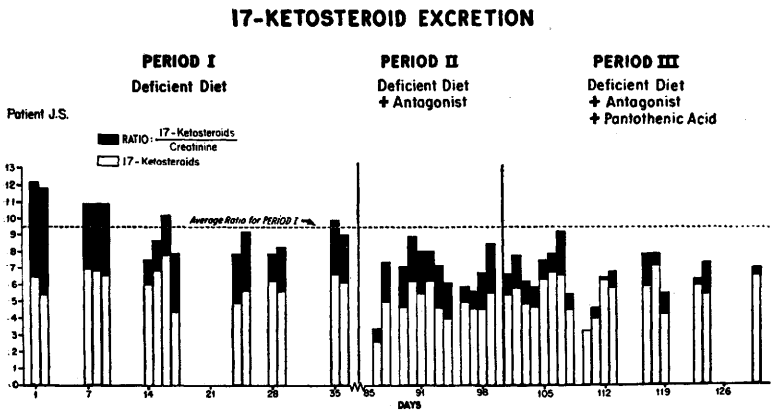

FIG. 2. Pt. J.S. Urinary 17 ketosteroid excretion.

of 17 ketosteroids and the defective diuresis after water ingestion. Similar events occurred in our first subject (Fig. 1, 2, and 3). One unexpected finding was gastric hypochlorhydria, and in one subject, histamine fast achlorhydria. These changes returned to normal during the recovery period. Many of our data are yet to be assembled and evaluated critically. The details of these observations will be the subject of a later report.

Discussion. From these studies, it is apparent that pantothenic acid, or its metabolic derivative, coenzyme A, is essential to the human economy. The spontaneous development of such a deficiency is unlikely since this vitamin is so widely distributed in foodstuffs, and is remarkably resistant to destruction by thermal or chemical agents. Development of an abnormal state by such artificial means is justified by the information derived therefrom. Apparently acetylation processes are essential to the integral function of the adrenal cortex, either in supplying cholesterol to this gland as a substrate, or in conversion of the substrate into steroid hormones, or both. What role acetylation plays in gastric secretion of hydrochloric acid is not apparent, but perhaps this is related to the functional level of the adrenal

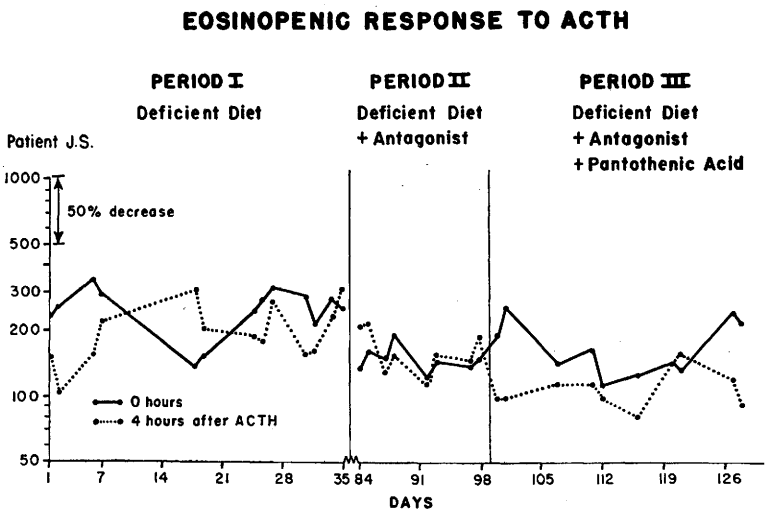

FIG. 3. Pt. J.S. Eosinopenic responses to ACTH were somewhat variable, but disappeared entirely during period of deficiency and returned partially when pantothenic acid was restored to the diet. Note that by using a semilogarithmic graph the distance between points remains constant for any given per cent of eosinopenic response regardless of initial count.

cortex since adrenal insufficiency is frequently accompanied by achlorhydria, and therapy with ACTH is accompanied by hyperchlorhydria. Finally, the development of peripheral neuropathy is similar to that in swine described by Wintrobe.

Summary. 1. An abnormal metabolic state has been induced in 4 human volunteers through combined use of a diet deficient in pantothenic acid and a metabolic antagonist, (omega-methyl pantothenic acid). 2. This abnormal state was accompanied by clinical and biochemical abnormalities suggesting adrenal cortical insufficiency, and by a peripheral neuropathy. 3. Administration of pantothenic acid alone did not immediately reverse the abnormal state. A good diet of natural foods and multiple vitamins resulted in rapid complete recovery.

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Sensitivity of Females of the C Stock to Male Infection with the Mammary Tumor Agent.* (21205)

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The mammary tumor agent (MTA), one of the factors usually required for the development of a high incidence of spontaneous mammary cancer in mice(8,9), may be administered by the injection of extracts of either normal or cancerous tissues from infected animals(10), including seminal vesicles(4) and cauda epididymis(15). Following the mating of agent-free females of some strains, which are either susceptible or resistant to the development of this type of cancer, with males of cancerous stocks, the appearance of mammary cancer in either the females or their progeny has been explained as resulting from the transmission of the MTA by the male at the time of coitus(3,7,11-17). Differences have been found in the sensitivity of agent-free females of various strains to become "infected," as well as the ability of males possessing the agent to infect females of either the same, or other stocks(12).

Females of the C (Bagg albino) stock have been found to be susceptible to the development of mammary cancer, as determined by a high incidence in mice which possessed the MTA, obtained by nursing females of a cancerous strain(1).

This report considers the sensitivity of

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