parin[1] was administered in doses ranging from 6 to 10 mg. The heparin was injected into a tongue vein as soon as sludge formation was noted following venous occlusion. In no instance was there any alteration in the nature of the sludge. However, it was noted that the clumps of sludged cells did not become adherent to the vessel wall as easily as in the control group. The sludged clumps built up in size until the vessel became almost occluded, but the mass did not become adherent to the endothelial lining. As a result, such masses rocked to and fro or flowed on in spurts. Occasionally such a mass, upon reaching a bifurcation, would split in two after being halted temporarily. Upon removal of the clamp, such vessels all resumed flow, and no thrombosis was observed.

Group III: Heparin administered before venous occlusion. Heparin was injected intravenously in doses ranging from .5 to 1 cc, depending on the weights of the dogs. This resulted in clotting times ranging from 15 to 28 min. After occlusion of the main vein, sludge formation was noted in each instance. Sludge appeared in from 9 to 35 min following occlusion. The stream slowed down in the usual manner but took considerably longer to stop completely than in the control series. In some instances there was considerable ebbing until the time of release. In the control series, as a rule, the majority of vessels were completely agglutinated within 30 min. In this series there was complete agglutination after 45 min in only one instance. In this case perhaps 25% of the vessels in the microscopic field were involved, the remainder maintaining patency. Except for this one instance, the sludged clumps of cells did not become adherent to the endothelial lining. Upon release of the occlusion, flow resumed in each instance, perhaps much more rapidly than in the control group.

It would thus appear that heparinization in clinically therapeutic dosages has no effect on sludge formation but will aid considerably in the prevention of thrombus formation around groups of sludged cells.

Group IV: Dicoumarol administered before venous occlusion. Six dogs were given adequate doses of Dicoumarol for a period of 3 days before the experiment. The doses ranged from 25 to 50 mg/day, depending upon the weights of the dogs. Prothrombin levels on diluted plasma, determined prior to occlusion, ranged from 15 to 20% of normal. Venous occlusions were made in the usual manner, and the small vessels were observed for sludge formation. Sludge appeared in each instance within 10–30 min. The appearance of the flow after approximately 1 hr was no different from that described in the heparinized animals. Again, the sludged masses did not become adherent to the endothelial lining and seemed to break up easily into smaller masses. In no case in this series was there any thrombus formation. Upon release of the occlusion, the blood within the small vessels resumed rapid flow.

Dicoumarolization therefore had no effect upon sludge formation but, as in the case of heparinization, prevented thrombus formation.

[1] Liquaemin, Roche-Organon, Inc., Nutley, New Jersey; 1 cc = 10 mg.

Our observations indicate that sludged masses of blood cells may serve as a matrix for thrombus formation, provided the other conditions favoring thrombosis are present. When anticoagulants are administered in doses known to be clinically effective, yet safe, thrombosis does not generally occur in the small vessels distal to an occlusion, but such doses do not prevent the formation of sludge.

The administration of anticoagulants prevents thrombus formation in the presence of sludge by preventing the sludged masses of cells from becoming adherent to the endothelial lining of the vessel. Although anticoagulants may cause some diminution in the tenacity of the adherence of the blood cells to one another, sludge formation, as such, is not prevented.

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An Experiment on Human Vitamin B_6 Deprivation[1]

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For some years there have been reports of the successful therapeutic use of pyridoxin hydrochloride in several disorders and disease symptoms, some of which have accompanied a state of malnutrition. Whether in these cases the action of the compound accrued from any nutrient property is difficult to judge, mainly because of the magnitude of dosage. Under such conditions a pharmacological rather than a physiological action of vitamins should be considered, and interpretations of nutritional significance should be weighted accordingly.

The nutritional need of several organisms, including some mammals, for vitamin B_6 compounds has been established, and something is even known of their probable functions in metabolic processes. There is no direct evidence, however, that any of them are nutritionally essential for man, even though the ordinary human dietary supplies them in relative abundance.

Ancel Keys wrote: ". . . the only evidence which we will accept as quantitatively exact on human requirements for B vitamins is that derived from controlled experiments on man. . . . The needs for controlled experimental studies on man are glaring" (*3*).

We are reporting an investigation on the possible human need for vitamin B_6.

An experiment was carried out on an adult male (W. W. H.), who subsisted upon a purified diet and re-

[1] Supported by a grant from the Division of Medical Research of the National Research Council of Canada.

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ceived all necessary known vitamins with the exception of B_6. The basal diet was a mixture of sucrose, corn oil, vitamin-free casein, mineral salts, and a cod-liver oil concentrate. Throughout most of the experimental period it was taken in an amount to supply daily 2,178 Calories, 58.5% of which were supplied by carbohydrate, 26.3% by fat, and 15.2% by protein. The unpalatability of the mixture made difficult the ingestion of a sufficient amount to supply the caloric needs of a moderately active man. At this level, however, it supplied 82.5 gm of protein and 50 mg of iron/day. Thiamin hydrochloride, calcium pantothenate, nicotinic acid, riboflavin, ascorbic acid, choline chloride, and inositol were taken in amounts to satisfy recommendations and in the proportions used in comparable work on rats. An extract of rice polish concentrate from which pyridoxin had been removed by treatment with Filtrol was also included. The results of the experiment are summarized in Table 1.

Throughout the experiment there was no evidence of a marked disturbance in nitrogen balance. If a little more than a gram is added to each figure for daily urinary nitrogen output, to allow for fecal nitrogen, a slightly positive balance is always apparent after adjustment to diet. This indicates no impairment in nitrogen assimilation. Blood total nitrogen, nonprotein nitrogen, and amino nitrogen were followed. In none was there any significant change, and only the values for nonprotein nitrogen are included in Table 1.

the 12th day of the B_6-free regimen. It remained at the one- to two-plus level for some time into the period of the ordinary diet. There was still a trace about 4 months afterward. A history of recurrent renal calculi complicates reference of this symptom to the vitamin deprivation.

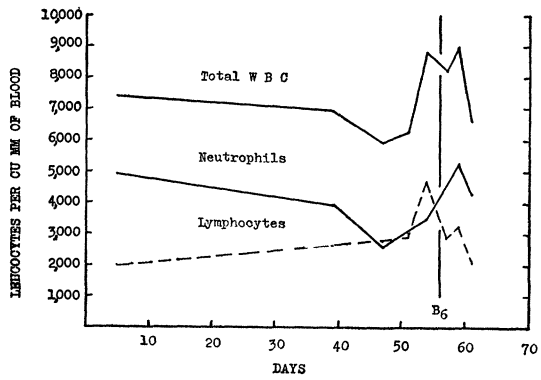

FIG. 1. The white blood cells during a human vitamin B_6-deprivation experiment. After 55 days without B_6 the diet was supplemented with pyridoxin hydrochloride.

Toward the end of the B_6-deprivation period the subject was aware of an unusual degree of depression and mental confusion, which disappeared very soon after supplementation of the diet with pyridoxin hydrochloride. No evaluation of this phenomenon is, of course, possible, but it appeared sufficiently real to engender the opinion that, in any work on human B_6 deprivation, provision should be made for the detection of mental and nervous symptoms.

TABLE 1

Regimen and exp. day*	Daily Calorie intake	Daily N intake (gm)	Daily urinary N output (gm)	Blood NPN (mg %)	Hb (gm %)	Leucocytes			Body wt. (lb)	Blood pressure (mm Hg)
						Total per mm³	L (%)	N (%)		
1 2 3										
0	2,905	15.75	14.00	33.6	16.0				129	130/90
5						7,400	27	67		
19			12.46	32.6	16.1					
20	2,178	11.81							130	120/82
38			13.60	32.5	15.4	6,900	38	57		
47					15.9	5,920	46	44	124½	
51						6,270	47	49	123½	
54			10.19	34.0	15.8	8,800	53	40	122	110/76
3						8,240	35	58	121	110/68
5			10.44	32.6	14.2	8,990	36	59		
7					14.8	6,600	31	65	120½	110/76
10									122½	120/70
38					15.1	7,720	35	60		
50					15.0				129	

* 1 = purified diet without B_6; 2 = purified diet with 10 mg of B_6/day; 3 = ordinary diet.

The initial hemoglobin value ranged between 16 and 17 gm %. During the B_6-deprivation period it reached a lower level and fell still lower during the period upon the basal regimen supplemented with pyridoxin hydrochloride. It remained at this level for 4 months after the resumption of an ordinary diet.

Weight and blood pressure were not improved during the period of B_6 supplementation, but were after an ordinary diet was resumed. Their fall, and the accompanying tiredness, perhaps had resulted from the low caloric intake.

An albuminuria was noted when a test was done on

The most interesting observation was an alteration in the white blood-cell picture, involving the lymphocytes and neutrophils, with no changes among the other cells. A few days after the addition of pyridoxin hydrochloride to the regimen the original white blood-cell picture returned (Table 1 and Fig. 1).

An increase in the proportion of neutrophils and a decrease in the proportion of lymphocytes have been reported among the white blood cells of monkeys showing pathological symptoms after some time on a B_6-free regimen (*4*). In work on humans there are reports of rather rapid increases in neutrophils after the daily intravenous administration of 50–200 mg of pyridoxin hydrochloride in some cases of pernicious anemia showing a low leucocyte count (*5*) and in cases of agranulocytic angina (*1*). This is particularly interesting with regard to our observation. Much more experimental work must be done, however, before any physiological relationship between vitamin B_6 and the human white blood-cell picture can be postulated.

Our experiment revealed that on a purified diet, over a period of about two months without vitamin B_6, no changes occurred which could unequivocally be considered as resulting from a lack of those compounds. There is the possibility, however, of albuminuria, of mental symptoms, and of white blood-cell changes.

A longer experimental period would have been desirable. In the case of monkeys on essentially the same regimen, months have been required to produce B_6-deficiency symptoms (*4*).

There is a strong possibility that human intestinal bacteria synthesize vitamin B_6-active compounds (*2*). In human experimentation of this nature an intestinal antiseptic might be used to advantage. A more attractive possibility is the use of the antivitamin desoxypyridoxin when more is known of its toxic properties.

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The Utilization of Carbon Dioxide by the Mature Rat in the Formation of Fatty Acids[1]

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This report is concerned with the incorporation of C^{14} from carbon dioxide in the saturated and unsaturated fatty acids of the rat. The fatty acids were derived from previous experiments (*1*) in which two unfasted rats weighing 624 gm (Rat I) and 473 gm (Rat II) were given $Na_2C^*O_3$ or CaC^*O_3, respectively, by intraperitoneal administration. The purified total fatty acids were separated into saturated and unsaturated fractions (*4, 5*). The fatty acids were decarboxylated in a stream of nitrogen (*2*), and the evolved CO_2 was collected in saturated barium hydroxide. The unsaturated fatty acids were converted to the solid calcium salts before counting. Tracer experiments ruled out the possibility that C^{14} could have been incorporated chemically or mechanically in the samples.

TABLE 1

RELATIVE SPECIFIC ACTIVITIES OF THE C^{14} IN THE FATTY ACIDS OF MATURE RATS ADMINISTERED C^{14}-LABELED CARBONATE*†

Sample	Rat I	Rat II
Total fatty acids	1.00 ± 0.1	1.00 ± 0.03
Carboxyl carbon of total fatty acids	.....	2.04 ± 0.3
Saturated fatty acids	1.47 ± 0.08	1.47 ± 0.05
Carboxyl carbon of saturated fatty acids	2.74 ± 0.3	2.66 ± 0.3
Unsaturated fatty acids	0.63 ± 0.08	0.80 ± 0.04

* The actual specific activity (specific activity = % of total administered dose/mg of carbon) of the total fatty acids of Rat I is 0.56×10^{-6} and of Rat II, 1.06×10^{-6}.

† The deviation of the results shown in the table is derived from the statistical error of the radioactivity measurements and was taken as equal to the square root of the sum of the squares of the standard deviation of the sample and background counts.

The data given in Table 1 show that (a) a very small fraction in the carbon of administered CO_2 is incorporated in the saturated fatty acids and, to a lesser extent, in the unsaturated fatty acids; and (b) the C^{14} content of the carboxyl carbon atoms of the saturated and total fatty acids is approximately twice as high as the average of all the carbon atoms in the respective fatty acids. It is of interest to note that the specific activities of the C^{14} in the glycerol portions of the fat molecules were about 10 times greater than those of the corresponding mixed fatty acids (*1*).

Similar results were found by Rittenberg and Bloch (*3*) following the administration to rats and mice of acetic acid labeled at the carboxyl carbon atom with C^{13}. They suggested that the C^{13} was present at alternate carbon atoms of the fatty acid chain, *i.e.* on the odd-numbered carbon atoms. Our results would indicate that the C^{14} activity derived from labeled CO_2 is also present on the odd carbon atoms of the fatty acids. The mechanisms through which this incorporation could take place can be derived from the interrelationships of the tricarboxylic acid cycle (*6*). Further evidence, particularly of the incorporation of the carbon of CO_2 into acetic acid, is needed to lend certitude to any one mechanism.

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[1] This work was supported by a grant from the Research Grants Division of the U. S. Public Health Service.

We are greatly indebted to Nathan Lifson, of the Department of Physiology, for his advice and criticism.

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