globulin present.[9] On the other hand, if a considerable amount of thrombin is released prior to the effect of the anticoagulant it may actually destroy part of the Ac-globulin. To guard against these difficulties the procedure described in this paper for drawing blood was followed carefully. Sodium citrate is also preferable to oxalate as an anticoagulant because Ac-globulin is more stable in stored citrated dog plasma than in oxalated plasma.

It is certainly possible, or even probable, that the effect of dicumarol on the coagulation mechanism is not restricted to prothrombin and Ac-globulin alone. Conflicting reports[10,11] have been presented as to the susceptibility of fibrinogen to dicumarol and a variation in serum antithrombin titer has also been proposed.[12] From the observations reported here it is seen that dicumarol has, in addition to its paramount function of lowering the prothrombin concentration, a secondary effect upon the coagulation mechanism through its reduction of Ac-globulin early in the period of dicumarol therapy. The definite drop in Ac-globulin which occurs following initiation of therapy should contribute at that time to the prevention of clot formation.

Summary. In human patients receiving dicumarol the plasma Ac-globulin level may be depressed by 20-50% following initiation of therapy. Individual variation was noteworthy. A gradual return to normal concentrations of Ac-globulin occurs within 3 weeks as therapy is continued and the prothrombin is maintained at a low titer. No appreciable difference from normal was found in the Ac-globulin values of patients who had been on dicumarol therapy for 1 to 14 months.

Dogs receiving larger dicumarol doses than were administered to human patients showed a similar Ac-globulin response and a more marked reduction in prothrombin. A period of slightly lowered Ac-globulin activity in the dog is followed by a temporary rise to levels above normal.

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16766

Indoleacetic Acid Studies in Man.*

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Corn products have usually been present in diets which have been associated with the etiology of pellagra in man,[1] blacktongue in dogs,[2] and nicotinic acid deficiency in rats.[3] It has been shown that the role of corn in the development of these deficiency syndromes is not due only to the low nicotinic acid content of the corn.[2,4,5] Other factors may be

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the low tryptophane content[5,6,7] and the possible presence of a "pellagragenic" agent[8,9] which could inhibit the utilization of nicotinic acid or the conversion of tryptophane to nicotinic acid.

The experiments on rats by Kodicek, Carpenter, and Harris[9] in 1946 suggested that the growth depressing or "pellagragenic" effect of corn may be due to its content of indole-3-acetic acid. In the present experiments the effect of indoleacetic acid supplements on nicotinic acid and tryptophane metabolism in man was evaluated by a study of the urinary excretion levels of nicotinic acid, tryptophane, and related metabolites, by subjects maintained on a controlled diet. While this work was in progress, other laboratories reported that indoleacetic acid had no effect on the growth of rats [10-12] or on the excretion of N'-methylnicotinamide (N'-Me) by rats.[12] Kodicek, Carpenter, and Harris[13] have also been unable to duplicate their earlier results with indoleacetic acid.

Experimental. The subjects were ward patients who were found to be free of complicating organic disease as determined by clinical and laboratory examinations. They were maintained in a separate metabolism ward and were permitted to be ambulatory. The wheat diet, which was used to maintain these subjects has been employed in other nicotinic acid and tryptophane studies.[7,14] This diet, which was planned to provide 2400 calories, contained 248 g of unenriched wheat products and supplied 43 g of protein, 430 mg of tryptophane, and 6 mg of nicotinic acid per day. Only 10% of the total protein was of animal origin. Variations in food intake, which were necessary in order to adjust the caloric intake, were recorded. After suitable control periods on these diets, indole-3-acetic acid (Eastman) was added as a supplement with dinner and supper.

Twenty-four-hour urines were collected in amber bottles containing 15 ml of glacial acetic acid, stored in the refrigerator and pooled in 48-hour periods for analysis of creatinine, nitrogen, thiamine, riboflavin, nicotinic acid, N'-Me, and tryptophane by methods previously described.[14] Since it has been observed that in rats and humans who had received tryptophane supplements, the apparent nicotinic acid content of the urine was markedly increased by autoclaving with strong acid,[14,15] the urines in two of the present experiments were autoclaved in the presence of 1 *N* H_2SO_4 for 30 minutes prior to assay of nicotinic acid. Another metabolite, which appeared in the urine of subjects who had received extra tryptophane, has tryptophane-like activity for *L. arabinosus* but is extractable from urine (at pH 4) by ether.[14] Ether extraction was tried in a few of the present experiments in an effort to detect any increase in this metabolite after indoleacetic acid administration.

Indoleacetic acid was estimated only in those urines obtained after administration of this substance, using the method suggested by Tang and Bonner.[16] In this test tryptophane gave 0.7% of the color obtained with indoleacetic acid while indole produced 14% and xanthurenic acid 1.3% of the indoleacetic acid color. About 10 to 15 mg of indoleacetic acid per day was found by this method in urines collected from the subjects during the basal period. Since many indole derivatives which appear in urine may interfere with this test, these basal values were not considered to be reliable.

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TABLE I.
Effect of Indoleacetic Acid Administration on Daily Excretion of Tryptophane, Nicotinic Acid, and Related Compounds by Subject I.

Diet per day	Days	Creatinine, g	Nitrogen, g	Thiamine, γ	Riboflavin, γ	Nicotinic acid, mg	N'-Me, mg	Tryptophane, mg
Wheat (2520 calories, 44 g protein)	1-2	1.1	5.9	71	470	0.5	1.6	11
	3-4	1.1	5.6	44	235	0.4	1.2	11
	5-6	1.2	5.4	50	150	0.5	1.4	13
	7-8	1.2	5.0	30	115	0.4	1.3	12
Wheat + 25 mg indoleacetic acid	9-10	1.3	5.8	33	107	0.5	1.6	13
	11-12	1.3	5.9	29	104	0.5	1.6	13
	13-14	1.2	5.7	20	53	0.5	1.2	14
Wheat + 50 mg indoleacetic acid	15-16	1.2	5.7	22	52	0.5	1.1	13
	17-18	1.2	5.8	21	52	0.5	1.5	14
	19-20	1.1	5.2	20	56	0.4	1.3	12
Wheat + 100 mg indoleacetic acid	21-22	1.2	5.3	19	65	0.5	1.2	15
	23-24	1.2	5.6	25	—	0.6	1.3	16
	25-26	—	—	—	—	—	—	—
Wheat	27-28	1.0	4.9	16	61	0.6	1.1	11
	29-30	1.1	5.5	21	80	0.6	1.3	15
	31-32	1.0	5.7	21	66	0.5	1.8	14
	33-34	1.1	5.8	27	74	0.5	1.8	15

TABLE II.

Effect of 200 mg of Indoleacetic Acid on Daily Excretion of Tryptophane, Nicotinic Acid, and Related Compounds by Subject I.

Diet per day	Days	Nicotinic acid			Tryptophane		Indoleacetic acid, mg
		As is, mg	Acid hydrol., mg	N'-Me, mg	As is, mg	After ether extr., mg	
Wheat (2530 calories, 44 g protein)	1-2	0.7	1.1	2.3	16		
	3-4	0.5	0.8	1.3	12		
	5-6	0.5	1.1	1.5	13		
	7-8	0.5	1.0	1.2	13	12	
Wheat + 200 mg indoleacetic acid	9-10	0.7	1.4	1.3	18	17	130
	11-12	0.6	1.2	1.4	18	17	138
	13-14	0.5	1.2	1.4	17		—
	15-16	0.6	1.7	1.9	16		83
Wheat	17-18	0.7	1.8	1.9	13		
	19-20	0.5	0.9	1.4	12		
	21-22	0.6	1.2	1.2	15		

TABLE III.

Effect of 200 mg of Indoleacetic Acid on Daily Excretion of Tryptophane, Nicotinic Acid, and Related Compounds by Subject II.

Diet per day	Days	Nicotinic acid			Tryptophane		Indoleacetic acid, mg
		As is, mg	Acid hydrol., mg	N'-Me, mg	As is, mg	After ether extr., mg	
Wheat (2370 calories, 41 g protein)	1-2	0.6	1.2	2.7	6		
	3-4	0.4	0.9	1.7	4		
	5-6	0.6	1.3	2.4	5		
	7-8	0.5	1.2	2.2	5	4	
Wheat + 200 mg indoleacetic acid	9-10	0.6	1.3	2.1	6	5	70
	11-12	0.5	1.2	2.1	6	5	97
	13-14	0.6	1.3	1.8	6		73
	15-16	—	—	—	—		—
Wheat	17-18	0.5	1.5	1.3	4		
	19-20	0.5	1.1	1.6	4		
	21-22	0.5	1.2	1.5	5		

Results. The values obtained in the analyses of the urine in these experiments are presented in Tables I, II and III. The subject for the experiments shown in Table I, and III was a 30-yr.-old male (W.H.) and in Table II a 47-yr.-old female (J.H.) The subjects were maintained on the basal wheat diet for 8 days before the supplements of 25 to 200 mg of indoleacetic acid were given. Since corn is reported to contain 20 to 100 mg of indoleacetic acid per kg[17,18] 25 mg of indoleacetic acid represents that found in 0.25 to 1.2 kg of corn while 200 mg of indoleacetic acid is equivalent to that obtained from 2 to 10 kg of corn products.

The creatinine and nitrogen excretion remained fairly constant in each experiment and are an indication of the completeness of urine collection and the constancy of the diet. The levels of thiamine and riboflavin excreted decreased rapidly, as has been found with other subjects on this diet,[14] and were not affected by the indoleacetic acid administration. For the sake of brevity, the above findings are shown only for the experiment in Table I.

The urinary nicotinic acid values obtained by microbiological analysis of the diluted urine were not significantly changed throughout the course of the experiments. The nicotinic acid values which were obtained after autoclaving the urine with acid (Tables II and III) also remained relatively constant. The slight increase in excretion of this metabolite at the end of the period of indoleacetic acid administration (Table II) is of questionable significance. Tryptophane is the only known compound which, when added to the diet, leads to an increase in excretion of this acid-hydrolyzable nicotinic acid-like compound.[14,15]

The excretion of N'-Me is at present the main index of nicotinic acid metabolism. In the present experiments there were no significant changes in excretion of N'-Me after administration of indoleacetic acid. In previous studies it has been shown that replacement of 190 g of the wheat of this diet by corn products resulted in a marked decrease in excretion of N'-Me.[7,14] This may have been due to the presence of an inhibitor in the corn,[8] or to the lower tryptophane content of the corn as compared with the wheat, or to a combination of these factors.

Tryptophane excretion increased slightly in all 3 experiments following the administration of indoleacetic acid and decreased to approximately the basal level after the supplementation was discontinued. The presence of indoleacetic acid in the urine could not account for the increased tryptophane values. Relatively high levels of indoleacetic acid (0.1 mg/ml) in the microbiological assay have been shown to have a slight stimulatory effect upon the utilization of tryptophane by *L. arabinosus*.[19] However, urines which were diluted for analysis of tryptophane, supplied less than 50 γ of indoleacetic acid for each 10 ml assay tube. Experiments in this laboratory show that 20 of 500 γ of indoleacetic acid per 10 ml assay tube has no activity for *L. arabinosus* in a test which can detect less than 0.2 γ of tryptophane and has no significant effect on tryptophane utilization. However, the metabolism of indoleacetic acid, either in the intestine or in the body, may lead to the formation of other compounds which can replace tryptophane or stimulate the utilization of tryptophane by *L. arabinosus*. Only 70 to 138 mg of indoleacetic acid were found in the urine after oral administration of 200 mg of indoleacetic acid (Tables II and III).

Extraction of some of the urines with ether showed (Tables II and III) that the increase in tryptophane excretion after indoleacetic acid supplementation was not due to the ether extractable tryptophane-like substance which is found in the urine after tryptophane administration.[14]

Discussion. In the present experiments the addition of up to 200 mg of indoleacetic acid per day to a low protein diet appeared to have no significant effect on nicotinic acid utilization or on the conversion of trypto-

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phane to nicotinic acid as evidenced by the excretion of nicotinic acid and N'-Me by man. Rosen and Perlzweig[12] showed that indoleacetic acid had no effect on the excretion of N'-Me by rats receiving a basal diet alone or with tryptophane supplements. Others have also been unable to obtain depression of rat growth by inclusion of indoleacetic acid in the diet, although the addition of corn or protein low in tryptophaone has retarded growth.[10-13] The replacement of a portion of the wheat diet by corn products has been shown to decrease the excretion of N'-Me by man.[7,14] This effect may be due to the lower tryptophane content of the corn as compared with the wheat or to the presence of an unidentified inhibitory agent in the corn.[8]

The increase in excretion of tryptophane compounds by man after the ingestion of indoleacetic acid may be due to the conversion of indoleacetic acid to a substance which has tryptophane-like activity for *L. arabinosus*. It is also possible that indoleacetic acid may have some effect on tryptophane metabolism in man which is separate from the tryptophane-nicotinic acid relationship. In rats, indoleacetic acid cannot replace tryptophane as a growth stimulant, when added to a diet deficient in tryptophane and nicotinic acid.[10-13]

Summary. The addition of 25 to 200 mg of indoleacetic acid per day to a diet low in protein and nicotinic acid appears to have no significant effect upon the utilization of tryptophane and nicotinic acid by man as evidenced by urinary excretion studies.

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Changes in Circulating Eosinophils in Man Following Epinephrine, Insulin, and Surgical Operations.*

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Forsham and his associates[1] have reported that pituitary adrenocorticotropic hormone (ACTH) produces a fall in circulating eosinophils which is dependent upon normal adrenal cortical function. Long and Fry[2] had previously demonstrated that epinephrine stimulates the adrenal cortex of the rat, and that this effect is mediated by the anterior pituitary. For these reasons we have studied the effects upon the circulating eosinophils of epinephrine, and later of insulin and of surgical operations. Studies have been made upon healthy persons, patients with adrenal and pituitary insufficiency, and patients with "diseases of adaptation"[3] such as essential hypertension and nonspecific colitis.

While this study was in progress, eosinopenia after intravenous epinephrine and insulin was reported by Godlowski,[4] and Thorn and his associates[5] reported that intravenous epinephrine reduced the eosinophil count in

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