octane-extracted DPNH-cytochrome c reductase in the Keilin-Hartree heart muscle preparation have confirmed the finding of Nason and Lehman(14) that this antioxidant is ineffective in replacing α-tocopherol. However, serum albumin was found to be an unsatisfactory agent for suspending DPPD in this system and other materials are being investigated.

Curative activity of DPPD with respect to muscular dystrophy in the rabbit is in contrast to its reported inability to prevent hypercholesterolemia and muscular degeneration in guinea pigs fed a tocopherol-low diet (15). An explanation of this apparent discrepancy must await further investigation.

Summary. Administration of DPPD to growing rabbits and rats fed a Vit. E-low diet does not modify rate of depletion of vitamin from the liver. Tocopherol content of skeletal muscle was essentially unaffected by dietary restriction. DPPD was as effective as α-tocopherol, at level administered, in bringing about a remission of muscular dystrophy and creatinuria in rabbits maintained on diet devoid of tocopherols. Ability of DPPD to partially regenerate sterile female rats maintained on Vit. E-free diet was confirmed. These findings indicate that the effectiveness of DPPD as a substitute for Vit. E is not due to conservation of the vitamin in tissues or to the presence of residual tocopherols in the diet.

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Conversion of Serotonin (5-Hydroxytryptamine) to 5-Hydroxyindoleacetic Acid by Rabbit Blood. (24486)

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Serotonin (5-hydroxytryptamine) is oxidized to 5-hydroxyindoleacetic acid (5-HIAA) in animals and man(1). The latter compound is excreted in the urine. The catalyst for oxidation of serotonin is thought to be monamine oxidase. Although many tissues contain this enzyme, liver is usually the most active source. Werle and Roewer(2) have reported an amine oxidase present in dog blood. In other species, however, blood has been considered to be lacking in this enzyme activity(3). Tabor *et al.* (4) found an amine oxidase in beef plasma, but substrate and inhibition specificities differed markedly from those described for the monamine oxidase in liver. Reserpine causes a release of serotonin and histamine from platelets *in vivo*(5). *In vitro* studies have shown that incubation of reserpine with rabbit platelets suspended in plasma also liberates serotonin and histamine. However, after in-

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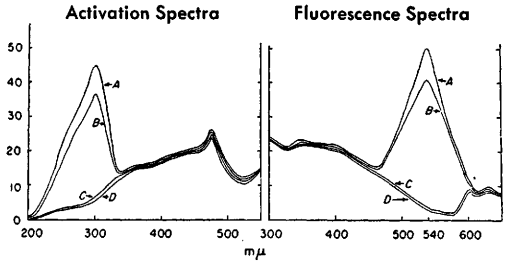

FIG. 1. Activation and fluorescence spectra in strong acid for 5-HIAA from (A) 3 ml of plasma after incubation of whole blood with reserpine, (B) 5 γ of authentic 5-HIAA, (C) 3 ml of control plasma, and (D) blank.

cubation of reserpine with rabbit whole blood, histamine was increased in the plasma, but serotonin was not.† Subsequent investigation has shown that serotonin is oxidized to 5-HIAA in presence of rabbit blood.

Material and methods. Blood was obtained from rabbits by cardiac puncture. All glassware used in handling blood or platelets was siliconized. The anticoagulant was disodium ethylenediaminetetraacetate (EDTA), 10% by volume of 1% solution in n-saline. Platelet rich plasma was obtained by the method of Dillard *et al.*(6). Red blood cells, suspended at bottom of centrifuge tube after complete removal of platelet layer by suction, were used for red blood cell studies. Although reduced in number, white blood cells, as shown by smear, were still present. Serotonin and histamine were determined following procedure of Weissbach *et al.*(7). By this method, both total 5-hydroxyindole compounds and also serotonin can be measured in the same blood or tissue aliquot. The 5-HIAA in plasma was assayed by modification of colorimetric method of Udenfriend *et al.*(1) for urine. The 5-HIAA was determined by measuring its fluorescence in 1 ml of phosphate buffer (pH 7) or after addition of strong acid to the buffer. Activation wavelength for 5-HIAA in both solutions is 300 mμ but the fluorescence wavelength in neutral or weakly acid solution is 350 mμ and in strongly acid solution is 540 mμ. In neutral or weakly acid solutions fluorescence is greater but less specific, with biologic materials, than when strong acid is used. In plasma, other material was found to fluoresce near 350 mμ at pH 7; at 540 mμ no interfering substance was apparent (Fig. 1). For all determinations of 5-HIAA in this study fluorescence was measured in strong acid at 540 mμ. Serotonin, as much as 100γ, did not produce a significant reading when assayed by the procedure for 5-HIAA.

Results. 1. Incubation of rabbit whole blood with reserpine. For experimental samples, 6 ml of blood were mixed with reserpine solution (20γ/ml in 5% dextrose in water) to make final dilution 0.3γ/ml; control samples were diluted with the same volume of 5% dextrose in water. After incubation at 37°C, whole blood determinations were done for total 5-hydroxyindoles, serotonin and histamine.

TABLE I. Total 5-Hydroxyindoles, Serotonin, and Histamine after Incubation of Rabbit Blood with Reserpine.

	Whole blood (γ/ml)					
	5-Hydroxyindole compounds				Histamine	
Incubation time (hr)	Control		Exp.		Control	Exp.
	Total	Serotonin	Total	Serotonin		
3.0	3.1	3.2	3.0	2.8	2.0	2.0
	4.2	4.3	4.1	3.8	2.2	2.1
3.5	4.1	4.0	4.0	3.5	1.2	1.2
	5.2	5.3	4.6	4.4	1.5	1.7
4.0	3.5	3.6	3.1	2.8	2.0	2.0
4.5	4.0	4.3	3.5	3.1	1.2	1.2
	4.1	4.1	3.5	2.4	1.6	1.5
5.0	4.6	4.8	3.7	2.9	3.1	3.0
	4.5	4.5	3.5	2.3	3.0	2.7
16.0	5.0	5.0	3.8	.2	3.7	3.4
	4.5	4.2	3.6	.3	2.1	1.8
	4.5	4.2	3.3	.9	3.0	2.7
	4.2	4.0	3.4	.8	2.1	2.4

† Unpublished observations.

TABLE II. Plasma 5-HIAA and Serotonin after Incubation of Rabbit Whole Blood with Reserpine.

Incubation time (hr)	5-HIAA (γ/ml) Plasma Control	Exp.	Serotonin (γ/ml) Plasma Control	Exp.	Whole blood Control	Exp.
5.0	.2	1.4	.2	.2	6.0	3.5
	.2	1.9	.1	.1	5.8	3.3
	.1	.7	.1	.1	3.5	1.8
	.1	1.2	.2	.2	5.0	2.7
16.0	.2	1.8	.2	.1	4.7	.4
	.2	2.5	.1	.1	3.3	.4
	.3	4.2	.1	.1	4.8	.9
	.2	2.5	.1	.1	2.4	.4

The results (Table I) show that amount of histamine/ml of blood was the same in control and experimental blood after incubation. However, the amount of serotonin in experimental blood was less than that in the control samples. The greatest difference was noted after 16 hours. Total 5-hydroxyindoles decreased slightly. Plasma content of serotonin was essentially the same in control and experimental samples (Table II). The evidence suggested that serotonin was liberated during incubation with reserpine and was converted to some other 5-hydroxyindole compound. When air over the blood was displaced by nitrogen, no change in final result was noted.

2. Assay for serotonin and 5-HIAA. Whole blood and plasma serotonin were determined, and in addition, plasma 5-HIAA. No 5-HIAA or other 5-hydroxyindole compounds except serotonin have been found in normal rabbit blood(8). The results given in Table II show that serotonin must be released and converted to 5-HIAA or a 5-HIAA-like compound. When whole blood was also incubated with serotonin (5γ/ml), with and without reserpine, for 5 hours, 5-HIAA was found in the plasma.

3. Identification of 5-HIAA. Fig. 1 gives fluorescence characteristics of authentic 5-HIAA, run through assay procedure, and of that isolated from plasma. The plasma from 10 ml of whole blood, incubated 5 hours with 1 mg of serotonin, was treated as for the 5-HIAA assay. However, the ether used for extraction was evaporated to a small volume and transferred to Whatman #1 paper for chromatography. The developing solvent was n-propanol 1 N amonia (5:1). After drying and spraying with 0.1 N HCl, pink fluorescent spots under ultraviolet light were seen for authentic 5-HIAA at R_f 0.21, for the experimental sample at R_f 0.22, and for standard serotonin at R_f 0.57. Ehrlich's reagent produced a blue green spot at R_f 0.21 for authentic 5-HIAA and at R_f 0.22 for the compound isolated from blood.

4. Part of blood responsible for oxidation. Platelets broken by shaking with water, normal rabbit plasma, and plasma from blood warmed with reserpine at 37°C for 12 hours were incubated as previously described(9) to determine presence of monamine oxidase activity. After 2 hours, the mixtures were assayed for serotonin and 5-HIAA. No conversion to 5-HIAA was found. Serotonin (2γ/ml) in n-saline adjusted to pH 7 with phosphate buffer and incubated, with or without reserpine, for as long as 12 hours, showed essentially no change in serotonin content. When serotonin, and histamine, were added to normal rabbit plasma, and incubated at 37°C 12 hours, destruction of both serotonin and histamine occurred (Table III). However, no conversion of serotonin to 5-HIAA was found by comparing amount of total 5-hydroxyindoles with the amount of serotonin present. Reserpine had no apparent effect on this reaction. When 5-HIAA was incubated with plasma for periods to 8 hours, no destruction of the acid was noted. Platelet rich plasma, incubated with serotonin 3 to 5 hours, with or without reserpine, did not yield 5-HIAA. Upon incubation of the red blood cell layer, obtained by differential centrifugation of blood, with serotonin (5γ/ml in n-saline) for 3 hours at 37°C in Dubnoff shaker, 5-HIAA

TABLE III. Incubation of Serotonin and Histamine with Normal Rabbit Plasma; Effect of Reserpine.*

Incubation time (hr)	Plasma (γ/ml)					
	Total 5-hydroxyindoles		Serotonin		Histamine	
	Reserpine	Control	Reserpine	Control	Reserpine	Control
0	3.6	3.6	3.7	3.6	3.1	3.0
1	3.3	3.4	3.6	3.4	2.5	2.6
3	2.7	2.7	2.6	2.5	1.9	1.7
6	2.0	1.9	2.1	2.2	1.6	1.5
12	.6	.7	.7	.6	.8	.7

* .3 γ/ml of plasma.

was found in the plasma. Addition of reserpine did not alter amount of final acid to any extent.

5. Marsilid (1-isonicotinyl 2-isopropyl hydrazide) *in vivo*. Marsilid is a potent inhibitor of monamine oxidase activity *in vitro* and *in vivo*(10). Blood was obtained from 3 rabbits before and 2 hours after being given 200 mg/kg of marsilid intraperitoneally. Blood was incubated with reserpine 5 hours, as before, and plasma analyzed for 5-HIAA and serotonin. The results for those samples drawn before marsilid injection were similar to results given in Table II. However, for those samples drawn after marsilid injection, control and experimental plasmas had the same average amount, 0.2γ/ml, of 5-HIAA, while average amount of serotonin in experimental plasmas was 1.0γ/ml and in control plasmas was 0.1γ/ml. These results indicate that serotonin was released by reserpine but not converted to 5-HIAA in the blood after marsilid treatment.

Discussion. In the present study, free serotonin was metabolized by rabbit whole blood. The product was thought to be 5-HIAA as shown by paper chromatography and by its fluorescence characteristics. Platelets did not oxidize serotonin to 5-HIAA. Both serotonin and histamine were destroyed slowly by normal rabbit plasma. However, serotonin was not converted to 5-HIAA by the plasma. Normal blood plasma has been stated to have an extremely low diamine oxidase activity(11), and diamine oxidase has been reported to attack monamines(12).

Serotonin, added to red blood cells separated by differential centrifugation from platelets, was converted, in part, to 5-HIAA. The red cell suspension was not entirely free of white blood cells. Consequently, red, or possibly white, blood cells must be involved in conversion of serotonin to 5-HIAA. Blood from rabbits pretreated with marsilid did not oxidize serotonin to 5-HIAA. The evidence suggested that the red blood cell, or possibly the white cell, contains an amine oxidase. Further evidence that the enzyme was probably a monamine oxidase was given by the fact that histamine was not destroyed in rabbit whole blood. Reserpine, except for its ability to release serotonin from platelets to be acted upon by the oxidative system did not appear to effect the conversion. The significance of a monamine oxidase in the cellular elements of blood is not known.

Summary. Free serotonin, in the presence of rabbit whole blood, is oxidized to 5-HIAA. The latter was identified by paper chromatography and by its fluorescence characteristics. Platelets or plasma do not produce the reaction. Evidence is presented that red blood cells, or possibly the white cells, are factors in the conversion of serotonin to 5-HIAA.

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Fluid and Electrolyte Movement Across Intestinal Wall of Bullfrog. (24487)

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While normal movement of fluid and electrolyte across intestinal wall is one of net absorption, experimental circumstances, both *in vitro* as well as *in vivo*, frequently do not result in absorption. The following results indicate that sacs of bullfrog intestine do absorb saline and establish concomitant gradients of chloride and bicarbonate ion.

Methods. Large bullfrogs (*Rana catesbiana*) were maintained in a state of pseudahibernation at 12°C. Sacs of mid-small intestine (jejunum) were formed by taking segments 2-3 cm in length from the pithed frog and tying each end. The large intestine was tied at the ends to form a sac. In half of the experiments, normal orientation of the sac wall was retained with mucosal surface bounding the lumen. The remainder of the sacs were formed after first everting the intestinal tube. The lumen of the everted sacs was thus bounded by the serosal surface. The sacs were incubated at room temperature (24°C) in 5 ml of a salt solution, pH 7.4, containing 87 mM NaCl, 20 mM $NaHCO_3$, 2.5 mM KCl, 1.25 mM $CaCl_2$, 0.5 mM $MgSO_4$, 1.33 mM Na_2HPO_4, 0.33 mM NaH_2PO_4 and 5 mM glucose/liter. About 0.3-1.3 ml of the same solution was placed inside each sac. 95% O_2 and 5% CO_2 was bubbled through Dubnoff shaker during experimental period of 4-6 hours. Water movement was measured by weighing the sacs at time "zero" (after 10-15 minute equilibration period) and at end of experiment, 4-6 hours later. Adherent water was removed from the sacs prior to weighing by rolling them on a dry glass surface. This resulted in a uniform stable weight. "Drying" the intestinal sacs by blotting them on a variety of papers did not result in a reproducible weight. Successive drying on filter paper was accompanied by small progressive decrease in weight. In early experiments, radio-inulin (C^{14}) was incorporated in the solution placed inside the sacs. Radio-inulin concentration within the sacs as well as in the bathing solution was determined(1) at end of experiment. The change of concentration of radio-inulin in the solution within the lumen of the normally oriented sacs indicated that weight change did measure net movement of water across intestinal wall. The bicarbonate concentration of inner contents of sacs was measured by back-titration, with NaOH, of an added volume of a standard HCl solution. Chloride concentrations of inner contents were determined by coulometric - amperometric method of Cotlove, *et al.*(2).

Results. The tabulated results indicate that there is a net movement, corresponding to absorption, of both water and electrolyte across wall of the normally oriented and everted sacs of small and large intestine.

Use of the everted sac has been favored in the study of isolated mammalian intestine because of the presumed better oxygenation of the epithelial surface(3). In our experiments, there was a significantly greater movement of water across the wall of the everted sac than across that of normally oriented sacs. However, while there was no movement of radio-

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