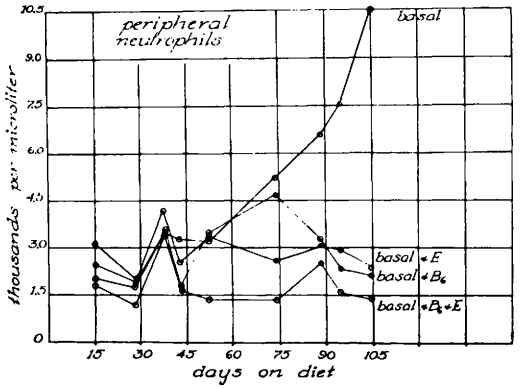

FIG. 1. Effects of vit. E and B_6 on peripheral neutrophils of rats.

mental day. This was approximately the same time the increased neutrophil levels became apparent. Creatinuria serves as a good indication of the beginning of nutritional muscular dystrophy in rabbits(10). Thus, rats deficient in both vit. E and B_6 behaved like rabbits deficient in vit. E, and granulocytosis may be a result of the metabolic alterations which instigate nutritional muscular dystrophy.

Summary. Sprague-Dawley rats deficient in both vit. E and B_6 exhibited a greatly increased peripheral neutrophil count. Supplementation of the diet with either of these vitamins prevented this increase. The B_6-deficient rats exhibited a slight lymphopenia which was not affected by vit. E. None of the animals developed anemia.

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Identification and Quantitative Determination of Serotonin (5-hydroxytryptamine) in Blood Platelets.*† (20855)

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The vasoconstrictor substance in blood serum was identified by Rapport and his coworkers as 5-hydroxytryptamine (serotonin) (1). Serotonin is not present in plasma(2), but appears in serum when clotting occurs in the presence of platelets(3,4). The fact that not only coagulation but also agitation, aging, freezing or exposure to ultrasonic frequencies cause the appearance of vasoconstrictor activity in platelet-rich plasma indicates that the clotting process *per se* is of no importance in the evolution of serotonin and suggests that the material is simply released from the platelets by these procedures. The occurrence of smooth muscle-stimulating material in crude platelet extracts supports this suggestion. In preliminary experiments, paper chromatograms of dog and rabbit platelet extracts were found to show spots which corresponded to 5-hydroxytryptamine(5). In the absence of information on the reliability of paper chro-

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matography for identification of this class of compounds, it seemed advisable to establish the identity of the material isolated by chromatography of platelet extracts by the simultaneous application of ultraviolet absorption measurements, colorimetry, and pharmacological assay. These experiments also permitted the determination of the quantity of serotonin in platelets and the examination of these cells for related substances.

Methods. The kindness of Dr. Seegers and his colleagues in making available to us dried bovine platelets was of immeasurable help. The platelets were separated from oxalated blood by differential centrifugation, washed once with about 3 volumes of oxalated saline, dried from the frozen state and sent to us(6). The properties of the platelet extracts were compared with those of standard serotonin solution (500 μg base/ml) prepared from synthetic 5-hydroxytryptamine creatinine sulphate.§ The standard, which was stable for days when kept in the icebox or freezer, was suitably diluted with saline before use. *Vasoconstrictor assays* were performed by a modification of the method of Page and Green(7) on the isolated perfused rabbit ear, by measuring the decrease in outflow of the perfusate after the injection of 0.1 ml into the arterial supply(8). Activity was also estimated from the contractions of the rat uterus isolated 18 to 24 hours after the injection of 10 μg/100 g of diethylstilbestrol. The *Folin-Ciocalteu reaction* was carried out as follows: To the sample in 2 ml of water, 0.5 ml of Folin Reagent (phosphotungstic acid) was added, followed by 1.5 ml of 15% aqueous Na_2CO_3. Two ml of water at 30°C were then added and the color read after 20 minutes in the Klett-Summerson colorimeter with a 66 filter.

Results. In preliminary experiments, 5 mg of dried bovine platelets were suspended in water (saline was equally effective) and refrigerated overnight. A considerable quantity of insoluble material remained. The activity of the supernatant as tested on the rabbit ear averaged 2.3 μg/mg dry platelets and was stable for at least a week in the refrigerator.

§ Kindly supplied by Dr. M. E. Speeter of the Upjohn Co.

To determine whether appreciable amounts of vasoconstrictor material remained in the insoluble residue, two experiments were performed in which the suspension of platelet material was subjected to ultrasonic vibrations for 5 to 15 minutes, producing a slightly opalescent solution. The pharmacological activity was not increased by this treatment, indicating that essentially all of the active material was obtained by overnight extraction.

For the chemical determinations, the extract was purified as follows. 51.8 mg of dry bovine platelets were suspended in 2.0 ml of water + 0.5 ml of acetone and placed in the refrigerator overnight. Then 38 ml of cold acetone were added. After standing in the refrigerator for 2 hours, the mixture was centrifuged in the cold at 900 x g for 30 minutes. The clear supernatant was removed and evaporated to dryness *in vacuo* with the aid of butanol and acetone. The residue was extracted with 5 ml of 95% aqueous acetone and the extract again evaporated to dryness *in vacuo*. The dry residue was taken up in 2 ml of water. This produced a flocculation of some lipid material. The mixture was centrifuged for 12 minutes at 20,000 x g. The supernatant solution (1.91 ml) was evaporated to dryness and taken up in 0.2 ml of 90% acetone. This solution was immediately placed on Whatman No. 1 paper in 5 individual spots, each representing 20 applications of 0.005 ml. Two control spots of 35 μg serotonin were applied to the same paper. Development was carried out by descending irrigation employing butanol saturated with N HCl(9) for 16 hours at 25°C. The solvent front had moved 30 cm. After drying the paper in air, one control and one extract strip were sprayed with p-dimethylaminobenzaldehyde(1) and dried at 105°C. Both showed a single spot with Rf = 0.15. The corresponding portions of the remaining four extract strips were combined and eluted with 4.5 ml of water at 37°C for 2 hours with shaking. The remaining control spot was eluted with 3.5 ml of water. Ultraviolet absorption spectra of these extracts are shown in Fig. 1. The deviations of the platelet extract curve from that of serotonin (slightly

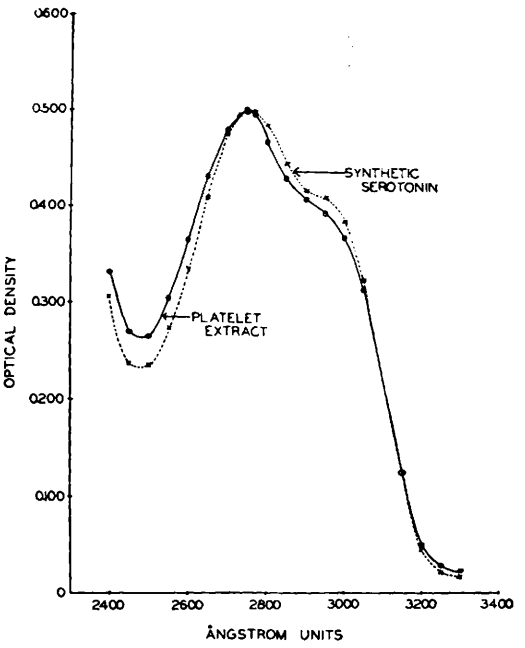

FIG. 1. Ultraviolet absorption spectrum of the substances eluted from the paper chromatogram: ⊙——⊙ Bovine platelet extract, pH 2.4. ×···× Synthetic serotonin eluted from the chromatogram $\left(\times\frac{502}{300}\right)$, pH 2.5.

increased absorption below 2700 Å and above 3200 Å, and slightly decreased absorption between 2800 and 3000 Å) indicate that a small amount of deterioration occurred during the manipulation of the material. These changes are exaggerated when elution of the developed chromatogram is delayed. More extensive decomposition produced a shift of the maximum to as low as 2700 Å with a loss in pharmacological activity of about 50%. The eluted extract had a 5-hydroxytryptamine content of 14.2 μg/ml calculated from the O.D. at 2750 Å ($\epsilon = 6200$), 14.2 μg/ml by Folin-Ciocalteu reaction, and 15 μg/ml by pharmacological assay on the rabbit ear and rat uterus. There is therefore no question that the material corresponds perfectly in properties to 5-hydroxytryptamine. The recovery of material, taking into account the volume losses during extraction and a recovery on elution from the paper chromatogram of 87%, corresponds to 98 μg of the base (1.9 μg/mg dry platelets), or 75% of that found in the crude platelet extract by pharmacological assay. In view of the instability of serotonin, this represents an excellent recovery.

No other indolic or phenolic substance was present on the paper chromatograms as determined by spraying with dimethylaminobenzaldehyde or with diazotized p-nitroaniline (9). This observation suggests that precursors of 5-hydroxytryptamine such as 5-hydroxytryptophane(10) do not occur in significant amounts in the platelet and must therefore be looked for in the megakaryocytes. The high percentage of the pharmacological activity of the crude extract which can be attributed to 5-hydroxytryptamine indicates that no other potent substance active on the vasculature of the rabbit ear or on the rat uterus is present in bovine platelets.

The platelet count of the slowly-centrifuged platelet-rich plasma employed as the starting material is maximally 1.1 x 10^6/mm^3 (estimated from the average values for bovine whole blood platelet count (684,000/mm^3) and hematocrit (40%)(11). Since about 4 g of dry platelets are obtained from 5 liters of this plasma(6), and since our assay has established that serotonin represents 2.3 μg/mg of dry platelets, it can be calculated that each platelet contains at least 1.7 x 10^{-9} μg of serotonin. The actual figure may well be several times higher because the yield of platelets in preparing the dry material is surely less than 100%. If all of the serotonin in beef serum (1.5 μg/ml)(12) is assumed to come from the platelets, it can be calculated that the amount of serotonin per platelet is 1.4 x 10^{-9} μg. This remarkably good agreement demonstrates that serum serotonin is entirely derived from the platelets, and that there is no reason to believe that plasma constituents play a role in its evolution other than by contributing to the breakdown of platelets during clotting.

Summary. Serotonin (5-hydroxytryptamine) was identified in bovine platelets by the coincidence of its ultraviolet absorption spectrum with that of synthetic serotonin, and by the agreement of analyses based on ultraviolet absorption, Folin-Ciocalteu reaction and pharmacological assay using the rabbit ear

and rat uterus. An average of 2.3 μg/mg of dry platelets was found, equivalent to at least 1.7×10^{-9} μg/platelet. This is more than sufficient to account for all of the serotonin found in bovine serum. No other indolic or phenolic substances were found in the platelet extracts.

Addendum. After this paper was in press, we learned that M. Bracco and P. C. Curti (*Haematologica,* 1953, v37, 721) had identified 5-hydroxytryptamine in rabbit and sheep platelets by similar methods.

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Experimental Aortic Disease in Albino Rats Following Renal Operations.* (20856)

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Striking changes in the aortae of male albino rats were observed at autopsies performed from one to 13 months following renal operations previously shown(1-4) to induce hypertension and renal disease. The aortae were increased in diameter and length, moderately tortuous in some instances and showed complete or incomplete ring-like bands of calcification giving them an appearance rather like trachea. The rats at the time of autopsy were from four to sixteen months old. Table I gives the incidence of grossly visible lesions in experimental and control rats. Since mild degrees of the aortic lesion were observed in one male and one female rat of a group of untreated animals previously set aside to age relevant data from this group have been included in the table.

Fig. 1 shows one of the more severely affected aortas. The bend pictured was present in the vessel during life so that it was appreciably displaced from the vertebral column. The lumpy, paler zones along the course of the aorta represent foci of medial calcification. Fig. 2 is a roentgenogram of this vessel. Fig. 3 is a magnified roentgenographic view of the same vessel compared with a control aorta from a rat of the same age. These diseased aortas appear very large in comparison with normal aortas after removal. Partly this is due to a genuine increase in size, attested to by curvature and beginning tortuosity, partly to failure of the diseased aorta to contract after removal.

Microscopic studies. Sections were stained with hematoxylin and eosin, by the van Gieson-Weigert and the von Kossa technics. The earliest stage of the lesion appeared to be associated with a deposition of finely granular basophilic material, identified as calcium salt by the von Kossa stain, in the spaces between the concentrically arranged elastic lamellae. Whether this deposition oc-

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