TABLE II. Time of Death, after Intraperitoneal Injection, of Mice Bearing the Ehrlich Ascites Tumor.

Mouse	Days after inj.
Control	7*
"	9
A	15
Control	16
A	16
A	16
B	16
B	16
B	18
A	18
B	20
B	20
Control	20
A	21
Control	26

* Killed. Used as seed for serial transfer.
A = Levan-cell mixture inj. immediately.
B = " " " " after 5 min.

the same with 2 to 5 injections (including the initial injection) of levan. The incidence of successful intraperitoneal transmission of the Ehrlich Ascites Tumor in CFW mice is greater than 98% in this laboratory: Of 271 mice used for its serial transfer not more than 3 unsuccessful transfers have been encountered and none since the forty-sixth transfer. Because of this high incidence of successful transfers, this form of the tumor was considered satisfactory for study of the effect of levan on tumor cells after direct mixing. Since survival times of the mice in the 2 experimental groups listed in Table II were not prolonged as compared to the controls, it would appear that the levan did not prevent the establishment of the ascites form of the tumor.

Summary. Injections of levan intraperitoneally into C58 mice did not alter the course of transmitted leukemia. Levan injected by tail vein or intraperitoneally failed to inhibit and did not apparently enhance establishment of a solid phase tumor at the site of inoculation of Ehrlich Ascites Tumor cells. The direct action of levan on the tumor cells injected immediately or after a 5 minute delay did not result in inhibition of either solid phase tumors or the ascites tumor.

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Platelets XVIII. Relationship of Platelets to Activity of 5-hydroxytryptamine Creatinine Sulfate.* (22615)

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It was originally thought that 5-hydroxytryptamine creatinine sulfate (5-HT) is an intimate constituent of platelets(1,2). This concept has more recently been revised to show that, like other factors(3), 5-HT may be only absorbed to platelets(4). However, the exact role of platelets in the metabolism of 5-HT remains controversial.

We have recently shown that 5-HT has a powerful local venopressor effect. Thus, measurement of local venous pressure is a simple and reliable method, detecting as little as 0.3 γ/5-HT administered in 1 minute time (5). In an effort to elucidate the relationship

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between 5-HT and platelets this technic was then applied to the study of the effects of incubation of 5-HT with various platelets preparations on the activity of the substance.

Materials and methods. Individuals with normal peripheral venous pressure were subject of this study. Changes in venous pressure were evaluated by means of the direct H_2O manometric method previously described (5). Crystalline 5-HT§ was diluted in saline solution prior to its use or its incubation with platelets, platelet products, plasma or serum. Human plasma was separated by centrifugation from whole blood collected in plastic bags containing standard ACD solution;‖ platelet-rich plasma was obtained by centrifuging whole blood at 500 rpm/30′/4°C; platelet-poor plasma by centrifuging at 3,000 rpm/30′/4°C. Serum was separated by centrifugation from whole clotted blood incubated for 6 hours at 37°C. Platelet suspensions in saline solution were prepared as previously described (6). Platelets, however, were washed only once to limit their damage and greatly concentrated so that platelets from 500 ml blood were resuspended in 15 ml of saline only. All fractions were obtained and used within 8 hours after collection of blood. Silicone-coated glassware, Arquad 2-C coated needles and plastic tubing were used in all experiments with platelets. Other technics used are described when appropriate.

Results. Preliminary observations showed that time of incubation was most important in determining the effect of various agents on the activity of 5-HT. Short and long incubation experiments were then run in each study. All experiments were performed in 4 different subjects. (a) *Venopressor activity of 5-HT after* short-time incubation *with fresh serum, platelet-rich plasma, platelet poor plasma and saline suspensions of platelets* (Fig. 1). One tenth gamma of 5-HT per ml was added to each preparation. A solution of 5-HT containing 0.1 γ/ml in physiological saline represented the control. Mixtures and control were incubated at 4°C for 30′. Fifteen

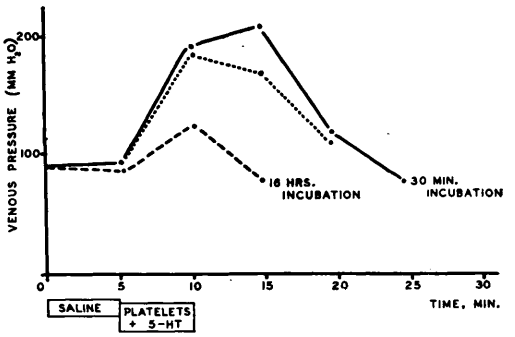

FIG. 1. Effect of short and prolonged incubation with intact platelets on venopressor activity of 5-HT.*

* A typical experiment. Platelet suspension was incubated with 5-HT for 30 min. or 16 hr at 4°C (see text). Dotted line shows response to the same dose of 5-HT by the same subject. Slight enhancement of venopressor effect of 5-HT through short incubation with platelets remains unexplained (see text).

ml of each preparation were then injected over a 5 minute period, at a speed of 3 ml/minute, one single subject receiving all preparations to reduce individual variability of results. Saline solution was given at the same speed between injections for periods of 15 minutes. *Fresh*¶ plasma, serum and suspensions of intact platelets had no venopressor activity. Saline solutions of 5-HT, fresh serum, fresh platelet-rich and platelet-poor plasma containing 5-HT kept full activity. Activity of 5-HT was enhanced after incubation with suspensions of platelets (Fig. 1). (b) *Venopressor activity of 5-HT after* prolonged incubation *with fresh serum, platelet-rich plasma and platelet-poor plasma* (Fig. 1). Mixtures of 5-HT and these various preparations were incubated at 4°C for 16 hours. None of the preparations tested showed venopressor activity. A control solution of 5-HT kept at 4°C for 16 hours had full activity. (c) *Interaction of 5-HT with various platelet preparations.* Once washed platelets from 1 pint of blood were resuspended in 15 ml of saline. Following incuba-

§ Kindly supplied by Dr. John Webb, Upjohn Co., Kalamazoo, Mich.

‖ Fenwal Laboratories, Framingham, Mass.

¶ Recent observations in this laboratory have indicated that *stored* plasma contains factors able to mimic the local venopressor effect of 5-HT.

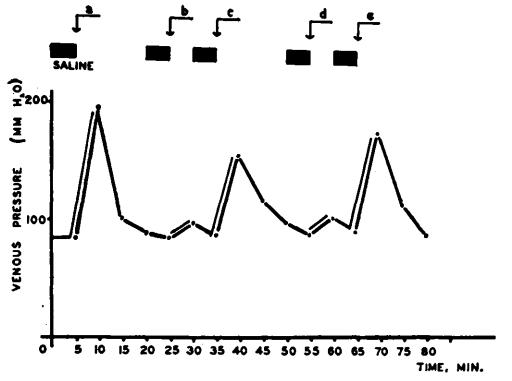

FIG. 2. Venopressor effect of various preparations.*

* (a) Saline solution of 5-HT (0.1 γ/ml); (b) intact platelets; (c) "platelet protein solution"; (d) supernatant, after incubation of 5-HT and platelets (16 hr); (e) "platelet protein solution" from platelets previously incubated with 5-HT.

Avg values from 3 sets of experiments supplied data for preparation of this composite chart. In actual experiments, longer intervals of time were used, and some experiments were carried out on different days.

tion with 1.5 γ of 5-HT for 16 hours at 4°C mixture was centrifuged at 3,200 rpm/30 min. 4°C. Supernatant was then transferred by suction to another container and platelets were resuspended by gentle shaking in 15 ml of fresh saline solution. Both supernatant and platelets failed to show venopressor activity (Fig. 2). To determine whether 5-HT had been entirely inactivated, destroyed, or, rather, incorporated by platelets, the following experiment was carried out. Two buttons of platelets were prepared simultaneously from 2 units of 250 ml of blood from the same donor. One was dissolved in 15 ml of physiological saline; the other in 15 ml of saline containing 0.1 γ/ml 5-HT (total 1.5 γ); both mixtures incubated for 16 hours at 4°C. In each instance, mixture was then centrifuged in the cold at 3,200 rpm/30', supernatant separated and platelets resuspended without washing in 1 ml of saline. Three ml of ethyl ether (which does not remove or destroy 5-HT) were added and mixture shaken vigorously for 5 minutes to obtain disruption of platelets(7). Mixture was then centrifuged at 3,200 rpm/10 minutes, supernatant ether discarded by suction, leaving behind a "*platelet-protein solution*". An additional reagent in this series of studies was saline solution containing 0.1 γ/ml. 5-HT, kept at 4°C for 16 hours.

All preparations were separately injected in healthy individuals with following results (Fig. 2): (a) 5-HT solution gave the expected venopressor response; (b) protein solution from platelets and platelets incubated with 5-HT gave a slightly less pronounced but still significant venopressor response.

Discussion. Fate and function of 5-HT are closely related to platelets, but the details of this relationship remain somewhat obscure. Since the mature platelet cannot form 5-HT from its precursor 5-Hydroxytryptophane (8), the substance may not be produced in platelets. Most commonly accepted theory is that 5-HT is produced in the enterochromaffine cells, then released into the blood stream. Absorbed by platelets, it is carried to various districts(9). Absorption by thrombocytes may represent a system of convection, as well as a mechanism of protection for 5-HT, since aminoxidases are present in plasma. If *in vitro* experiments with synthetic 5-HT duplicate the behavior of natural substance *in vivo,* our studies elucidate certain aspects of the relationship between 5-HT and platelets. Short time incubation with fresh serum, plasma and platelets did not inactivate 5-HT; long term incubation did. In case of plasma and serum, degradation of 5-HT may have occurred on long incubation; in the case of platelets an alternative explanation offered itself, absorption or binding of 5-HT by thrombocytes. This hypothesis received support from some of our findings, since, following prolonged incubation with 5-HT, intact platelets did not exhibit venopressor effect; products of disrupted platelets did. Therefore, whatever the nature of absorption or binding between platelets constituents and 5-HT, this was a strong one requiring destruction of the integrity of the platelet body. A complicating feature of these studies was the observation that "platelet protein solution" a product of platelet disruption showed significant venopressor effect, without addition of or incubation with 5-HT. This suggested that platelets contain an appreciable amount of

natural 5-HT or, perhaps, of other venopressor agents. Protein solutions from platelets incubated with 5-HT, however, proved to possess higher venopressor effect than from platelets alone, all experimental conditions being equal. Presumably, some 5-HT had been absorbed or bound by platelets.

Summary. 1. 5-hydroxytryptamine creatinine sulfate (5-HT) administered intravenously causes significant elevation of the local venous pressure at doses as small as 0.3 γ/min. This effect is not affected by short-time incubation with fresh serum, platelet-rich and platelet-poor plasma. Incubation for 16 hours causes destruction of 5-HT activity. 2. Activity of 5-HT also disappears after incubation for 16 hours with suspensions of washed platelets. Some of the activity, however, can be recovered after total disruption of thrombocytes. Also a protein solution from human platelets exhibits significant venopressor activity, while intact platelets are inactive. 3. These findings suggest: (a) presence of natural 5-HT or of substances with venopressor effect in human platelets; (b) absorption of 5-HT by platelets upon incubation; (c) strong bondage between 5-HT and platelet constituents.

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Action of Sodium Salicylate on Tissue Gas Tensions.* (22616)

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It is known that after intravenous administration of sodium salicylate (100 mg/kg body weight) ventilation increases secondary to both direct respiratory center stimulation and elevation in metabolic production of CO_2; alveolar CO_2 tension decreases 10%; oxygen consumption increases approximately 70%; arterio-venous oxygen content difference increases by 50%; and cardiac output is elevated by 20%(1).

With the above actions in mind, the question arises as to how tissue gas tensions are altered. Tissue carbon dioxide may be expected to follow alveolar air, but the oxygen tension in tissues is determined by an exacting relationship between the tissue metabolic demand and its blood supply. The problem of measuring gas tensions in tissue was approached through construction and analysis of subcutaneous depots of gas, a technic developed by Campbell(2) and shown to provide an adequate estimate of extracellular, if not intracellular fluid gas tensions.

Methods. Gas depots were formed in the interscapular region of non-fasting albino rats by subcutaneous injection of 20 cc of sulphur hexafluoride, an inert gas of high molecular weight and low blood solubility. Its prolonged disappearance time facilitated sampling and in no way interfered with the equilibrium tensions of CO_2 and O_2(3). Twenty-four hours after depot construction, 2 groups

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