of 9.0 Mv. with and 6.7 Mv. without sucrose exchange. Here, then, is evidence that spreading depression is not an all or none phenomenon. It can occur at the electrode nearest to the stimulators without propagating to the postcentral gyrus and when propagation does occur the amplitude of the reaction can vary in terms of the "D. C." millivolt swing.

There is some indication in our records that the brain wave amplitude decreases after exposure to static sucrose and increases somewhat when the sugar is continuously flowed through the pool. This effect could well be explained by electrolyte entering the stationary sugar medium from cerebrospinal fluid or brain and creating a partial shunt resulting in an apparent decrease in brain wave amplitude.

Therefore, substitution of Ringer-Locke solution for sucrose results in almost complete shunting of the electrocorticogram, creating a nearly isoelectric recording. In many experiments the above procedure caused discrete spikes to appear, especially on E_1, so that an otherwise isoelectric recording was punctuated at varying intervals by low amplitude oscillations.

In the last 2 experiments, the Ringer-Locke solution was permitted to flow continuously instead of remaining static during the period prior to control stimuli. Whereas 40% of the stimuli given after static Ringer-Locke exposure produced no response, 63.6% of the stimuli after flowing Ringer-Locke treatment yielded no type of depression. This result indicates that sucrose is not removing a non-electrolyte extractive but rather is attracting one or more of the ions present in the subarachnoid space and replaceable by the Ringer-Locke solution. This interpretation seems tenable, since exposure to the buffered physiological salt diminishes the incidence of spreading depressions and increases the number placed in the "no type of depression" category.

Discussion. The mechanism by which the sugar solution increases the incidence of propagated depressions remains to be conclusively demonstrated. Exposure to the physiologically balanced ion solution following the dialytic method appeared to decrease the incidence of spreading depression to a significant extent. This result suggests that the removal of one or more of the ions present in the subarachnoid space creates a deficient chemical environment conducive to an increased incidence of the spreading depression phenomenon. The postulated deficiency could be due to a simple reduction in electrolyte concentration or the inhibition of an enzyme system by the loss of an ion activator.

Summary. Exposure of the rhesus monkey's cerebral cortex to a flowing 9.95% sucrose solution significantly increases the incidence of the spreading depression reaction evoked by electrical stimulation.

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Relationship of Coagulase-Globulin to Prothrombin. (18221)

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In previous reports(1,2) we have shown that *staphylocoagulase* reacts with a plasma globulin factor, *coagulase-globulin,* to form a thrombin-like substance, *coagulase-thrombin. Coagulase-globulin* is, by itself, unable to clot pure fibrinogen. The reaction of *staphylocoagulase* with *coagulase-globulin* to form the active clotting agent yields hyperbolic time-concentration and conversion curves, similar to those obtainable for the conversion of prothrombin to thrombin. Because of other similarities, as well as some differences, in their action, it seemed necessary to investigate what part, if any, pro-

1. Miale, J. B., *J. Hematol.,* 1949, v4, 1039.
2. Miale, J. B., *Blood, J. Hematol.,* 1949, v4, 1317.

thrombin plays in the staphylocoagulase reaction, and to determine the relationship of *coagulase-globulin* to prothrombin and to prothrombin activity.

Experimental. I. *Methods.* (a) *Coagulase-globulin (CG) titer*, expressed as the reciprocal of the highest dilution of oxalated plasma giving 4+ coagulation of a system composed of 0.5 cc of tested material in serial dilution in imidazole buffered saline pH 7.4 + 0.5 cc of 2% fibrinogen (Fraction I-2A) + 0.1 cc of standard staphylocoagulase(1). It should be noted that only fibrinogen free of CG may be used in this titration. (b) Prothrombin activity, according to the method of Warner, Brinkhous, and Smith(3) as modified by Hurn and Mann(4). (c) All determinations carried out at 35°C. (d) All plasma centrifugations in the cold at 3000 r.p.m. for 30 minutes.

II. *Materials.* (a) *Fresh plasma:* from 4.5 cc of human blood plus 0.5 cc 0.1 M sodium oxalate, by centrifugation at 3000 r.p.m./30 minutes. (b) *CaF_2 plasma*: 0.3 cc of M/CaF_2 suspension is centrifuged strongly and the supernatant fluid completely drained away. The sediment is mixed with 1 cc of plasma, incubated with intermittent stirring at 37°C for 15 minutes, then centrifuged. Occasionally it is necessary to repeat this procedure twice in order to remove the last traces of prothrombin. (c) *Defibrinated plasma*: by the addition of thrombin (Upjohn), 15 units/cc of plasma. (d) *Defibrinated CaF_2 plasma*: plasma from (c) treated with CaF_2 as in (b). (e) *$Ca_3(Po_4)_2$ plasma*: 1 cc of fresh plasma added to centrifuged sediment from 1 cc of freshly prepared and thoroughly washed 0.008 M suspension of $Ca_3(Po_4)_2$, incubated for 10 minutes at 37°C, then separated by centrifugation. (f) *$Al(OH)_3$ plasma*: fresh plasma to which is added an equivalent of 1% freshly prepared and thoroughly washed $Al(OH)_3$, incubated at 37°C for 15 minutes, then separated by centrifugation. (g) *$BaSO_4$ plasma*: 100 mg of $BaSO_4$/cc of plasma, stirred for 15 minutes at 37°C, then separated by centrifugation. (h) *Seitz plasma*: fresh plasma filtered through Seitz filter (EK Germicidal Pads). Five filtrations of 100 cc of plasma through at least 3 pads usually adequate to remove all prothrombin.

TABLE I. Effect of Adsorption on Coagulase-Globulin Titer and Prothrombin of Oxalated Human Plasma.

Plasma	CG titer	Prothrombin units
Fresh (control) (a)	10^4	330
CaF_2 (b)	10^4	0
Defibrinated (c)	10^4	343
Defibrinated CaF_2 (d)	10^4	0
$Ca_3(Po_4)_2$ (e)	0	0
$Al(OH)_3$ (f)	0	0
$BaSO_4$ (g)	0	0
Seitz (h)	10^5	0

Results. As shown in Table I, human oxalated plasma can be freed of prothrombin by adsorption with CaF_2, but this treatment does not affect the coagulase-globulin. On the other hand, the other adsorbants, $Ca_3(Po_4)_2$, $Al(OH)_3$, and $BaSO_4$, are active adsorbants of both coagulase-globulin and prothrombin.

A second dissociation of prothrombin and coagulase-globulin can be demonstrated by filtration of plasma through a Seitz filter. Successive filtrations reduce the prothrombin content to variable degrees(5), but do not affect the coagulase-globulin titer.

Discussion. A dissociation between coagulase-globulin and prothrombin can be demonstrated by means of adsorption on CaF_2 as well as by repeated filtrations through a Seitz filter. This, plus the previously described(2) differences between the activity of prothrombin and of the staphylocoagulase system in the presence of substances having anti-prothrombin and anti-thrombin activity (Chlorazol fast pink, oxalates, citrates, soluble fluorides, and heparin) indicates that the conversion of *coagulase-globulin* to the clotting agent *coagulase-thrombin* is independent of prothrombin and of prothrombin activity, and that coagulase-globulin is not iden-

3. Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, v114, 667.

4. Hurn, M., and Mann, F. D., *Am. J. Clin. Path.*, 1947, v17, 741

5. Tager, M., and Hales, H. B., *J. Immunol.*, 1948, v60, 1.

tical with prothrombin.

Summary. A further dissociation between coagulase-globulin and prothrombin has been demonstrated by means of adsorption on CaF_2 and by repeated filtrations through a Seitz filter. This indicates that coagulase-globulin is not identical with prothrombin.

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Ovarian and Lymphoid Tumors in Female Mice Subsequent to Roentgen-Ray Irradiation and Hormone Treatment.* (18222)

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Ovarian tumors of the granulosa cell type have developed in mice subjected to total body irradiation(1) with roentgen rays and after intrasplenic transplantation of ovaries into castrated mice(2). The development of ovarian tumors under the latter experimental conditions has led to the hypothesis that prolonged exposure to excessive amounts of gonadotrophic hormone of the pituitary constitutes a carcinogenic stimulus(3). Ovarian tumors did not appear when estradiol benzoate or testosterone propionate were injected into mice with intrasplenic ovarian grafts. Furthermore, preliminary experiments indicated that gonadal hormones prevented the appearance of ovarian tumors in mice subjected to roentgen irradiation(4).

Many investigators, since the original observations of Furth(5), have noted the leukemagenic action of x-rays in mice and more recently the effects of additional treatment with estrogens(6).

Materials and methods. Three groups of 50 female mice of the BC† stock were used in the following experiments. The groups were so constituted that littermates were distributed throughout all groups as uniformly as possible. At ages ranging from 34 to 92 days the mice received a total body irradiation of 280 to 380 r. An equal number of mice in each of the groups received comparable doses. From 42 to 45 animals in each group survived irradiation, the earlier periods of the experiment and are included in the following data. The animals were maintained in an air conditioned room (72-74° F temperature and 50-60% relative humidity). They were fed a commercial food (Purina Fox Chow) and water *ad libitum*. The mice were examined daily throughout the course of the experiments. One group received weekly subcutaneous injections of 0.05 cc of sesame oil, one group 1.25 mg of testosterone propionate‡ dissolved in 0.05 cc of sesame oil; and one group 16.6 μg of estradiol benzoate§ dissolved in 0.05 cc of sesame oil. Injections were omitted on rare occasions in

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2. Li, M. H., and Gardner, W. U., *Cancer Res.*, 1949, v9, 35.

3. Gardner, W. U., *Cancer Res.*, 1948, v8, 397.

4. Li, M. H., Kaplan, H. S., and Gardner, W. U., unpublished.

5. Furth, J., *Am. J. Roentgenol.*, 1934, v32, 377.

6. Kirschbaum, A., Shapiro, J. R., and Mixner, H. W., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 632.

† BC refers to mice obtained as follows (PM♀ x C_3H♂) (F1♀ x PM♂) and subsequent brother to sister inbreeding. "PM" refers to mice sent to the writer by Dr. H. B. Andervont and derived from the colony of Drs. Pybus and Miller in England. The mice used in the present study were from the eleventh to the fifteenth generations of inbreeding—and largely from the twelfth to the fourteenth.

‡ The testosterone propionate was obtained from Organon, Inc., through the courtesy of Dr. Kenneth Thompson.

§ The estradiol benzonate was obtained from Ciba Pharmaceutical Products, through the courtesy of Dr. E. Oppenheimer.