release from the anterior pituitary(14).

3. (And also apparent from Fig. 1) the extent of daily change in brain 5-HTA concentration is relatively small as compared with the amplitude of 24-hour changes observed under standardized circumstances for some other body functions of rodents(2,15). The suggestion by Pletscher, Shore, and Brodie(10) that 5-HTA exists mainly in a bound form comes to mind in this connection. Before this assumption is validated, the possibility that those changes noted herein may reflect primarily changes in free 5-HTA can hardly be discussed. It also must be remembered that 5-HTA behavior of the entire brain was studied herein. Thus, drastic changes in certain parts of the brain, like the hypothalamus, may be obscured if other parts of the brain do not vary at the same time in the same direction. Analysis of these problems must await the study with more sensitive procedures(8) of the behavior along the 24-hour time scale of 5-HTA concentration in various areas of the brain.

Summary. ZBC mice, male or female, 5 or 7 weeks of age, were studied under conditions standardized for evaluation of 24-hour periodicity. A small but significant decrease in 5-HTA concentration of brain pools was noted prior to usual time of arousal. A sex difference in brain 5-HTA concentration also was recorded, the females exhibiting slightly higher values.

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Metal Chelates as Therapeutic and Detoxifying Agents in Gas Gangrene.* (22587)

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The ability of ethylenediamine tetraacetic acid (EDTA) to protect mice against a lethal dose of *Clostridium perfringens* Type A toxin has been noted(1). It was shown that this protection was due to the metal chelating ability of EDTA inasmuch as it was reversed by Zn^{++}, Co^{++} and Mn^{++}. In those experiments the toxin and EDTA were mixed together before injection but it appeared that EDTA could also protect when given independently of the toxin. In relatively large doses EDTA is toxic for animals because it induces hypocalcemia, and thus large amounts cannot be administered safely. Although Ca^{++} activates the principal lethal component of this toxin, a lecithinase, it was demonstrated that it did not reverse the protective effect of EDTA. This observation suggested that the calcium chelate of EDTA (Ca-

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TABLE I. Protective Effect of CaEDTA in Mice Injected with *Cl. perfringens* Toxin.

Conc. CaEDTA inj. (%)	No. survivors/ No. animals inj.
5	10/10
2.5	8/10
1	7/10
.33	3/10
0	0/10

EDTA), which is non-toxic, may be of value in protecting against the toxin, and also perhaps as a therapeutic agent in gas gangrene. *Cl. perfringens* is the organism most frequently involved in gas gangrene and its virulence is thought to be due to a battery of enzymes which aid it in invading tissues. It was hypothesized that if CaEDTA inhibited all, or some of these enzymes, the invasiveness of the organisms would be curbed and the infection would be stopped. In this report evidence that CaEDTA does protect animals against a toxic culture filtrate of *Cl. perfringens* and experimental gas gangrene is presented.

Procedures and results: *Protection against a toxic culture filtrate*: It was determined that 0.5 cc of a 1:4 dilution of a toxic culture filtrate of *Cl. perfringens* Type A† contained 2 M.L.D. when injected intracutaneously (I.C.) into 20-25 g white mice. All mice died within 24 hours with this dose of toxin. The CaEDTA used was a purified preparation dispensed for the treatment of lead poisoning.‡ All dilutions of the CaEDTA and toxin, and in later experiments the cultures and EDTA, were made in sterile 0.15 M NaCl. Five-tenth ml of toxin was injected I.C. into one side of the abdominal surface and at the same time 0.5 ml of the CaEDTA solution was injected I.C. into the abdominal surface on the other side of the mouse. The results are given in Table I. The survivors given toxin plus 5% or 2.5% CaEDTA manifested normal activity and no sign of illness, while those surviving with 1% and 0.3% CaEDTA were ill for a few days before resuming their normal activity.

Protection against experimental gas gangrene. These experiments were initiated on an exploratory basis but the results are so definitive that they are deemed worthy of being reported at this time. Lack of animal facilities prevented the use of larger numbers of animals, thus there is a variation in the number of animals in some experimental groups and questions relating to the optimum therapeutic utilization of CaEDTA remain to be answered. A strain of *Cl. perfringens* isolated from a patient with gas gangrene§ was grown in a medium composed of 3% Trypticase Soy Broth and 1% Proteose Peptone #2. An infection was initiated by injecting a dilution of a 5 hr culture into the thigh muscle of a guinea pig. CaEDTA was administered by subcutaneous injection of a 5% solution into the axillary region. The dose was 30 mg/100 g and was equally divided between each axilla. The initial injection of CaEDTA was made at the same time the culture dilution was injected. When more than one injection of CaEDTA is recorded they are each at this dosage level at approximately 12-hour intervals. In some experiments the culture was diluted either in 1% CaEDTA or 1% EDTA‖ and the volume of these suspensions injected was the same as that recorded for the culture dilution. These latter solutions were adjusted to pH 7.3 before the cultures were diluted in them and it was determined that they were not bactericidal in these concentrations. The different groups of animals in an individual experiment were injected with aliquots of the same culture dilution within the time interval necessary to carry out the injections. The results of the experiments are recorded in Table II.

Comments. In Exp. I, the animals in Groups B and D were slightly ill, except for the one fatality which became ill on the third day. Except for Group D in Exp. III, none of the treated animals in Exp. II and III

† Kindly supplied by Lederle Laboratories Division, American Cyanamid Co.

‡ Sold under trade name of Versenate. It is the calcium disodium salt of EDTA. Kindly supplied by Riker Laboratories, Inc., Los Angeles, Calif.

§ "Geiger" strain. Obtained from Laboratory of Dr. John Maclennan.

‖ Disodium Dihydrogen Versene, analytical reagent. Kindly supplied by Dow Chemical Co.

TABLE II. Effect of CaEDTA and EDTA on Experimental Gas Gangrene.

Exp.		Dilution of culture inj.*	Treatment	No. animals in group§	Time of death (No. dead in hr) 24	48	72	96	120	No. survivors	Extent of lesion of survivors‖ (No. in each category) A	B	C	D	E
I	A	1:10	None	2	2	—	—	—	—	0	—	—	—	—	—
	B	"	5 inj. CaEDTA	4	0	0	0	0	1	3	0	0	3	0	0
	C	1:100	None	2	2	—	—	—	—	0	—	—	—	—	—
	D	"	5 inj. CaEDTA	4	0	0	0	0	0	4	0	0	4	—	—
II	A	1:50	None	8	2	1	1	1	0	3‡	0	0	0	0	3
	B	"	3 inj. CaEDTA	8	0	0	0	0	0	8	4	3	1	0	0
	C	"	1 " "	6	0	0	0	0	0	6	3	0	3	0	0
	D	"	Culture dil. in EDTA	5	0	0	0	0	0	5	1	1	3	0	0
III	A	1:20	None	10	5	4	1	—	—	0	—	—	—	—	—
	B	"	1 inj. CaEDTA†	10	0	0	0	0	0	10	7	2	1	0	0
	C	"	Culture dil. in EDTA	4	0	0	0	0	0	4	1	0	0	1	2
	D	"	" " " CaEDTA	4	0	0	0	1	1	2	0	0	0	0	2

* 0.5 ml in Exp. I; 0.25 cc in Exp. II and III.

† Three animals given 2nd inj.

‡ Unlike other survivors, who were recovering, these had gaping holes in their sides and were moribund (see text).

§ Wt range of animals: Exp. I, 750-900 g; Exp. II and III, 300-500 g.

‖ Extent of lesion: Lesion recorded is maximum that developed (see text). A. Slight or no swelling at inj. site. B. Definite swelling at inj. site. C. Swelling involves most of thigh. D. Swelling involves rear quadrant. E. As in D, but with gas spreading up side of body.

became ill, in spite of the lesions that developed in many of them. The lesions of treated animals recorded as A, B and C in Table II developed to that extent within the first 24 hours after injection, and started to subside after that time. At the end of the 5-day experimental period most of them were healed. The lesions of treated animals recorded as D and E developed to that extent in the course of about 4 days, then started to subside.

Discussion. Inasmuch as the toxins of *Cl. perfringens* are thought to be responsible for the ability of the organism to invade tissues and cause death, the fact that CaEDTA protects against a lethal dose of the toxin suggests that it protects against experimental infection by inhibition of one or more of the toxins that are produced. Although only a small number of animals were involved, when the organisms were suspended in either CaEDTA or EDTA the latter appeared to have a greater protective effect. These results do not detract from the effectiveness of CaEDTA when given systemically in view of the marked differences in dosage used with the 2 methods of administration. They do suggest, however, that the optimum therapeutic approach may be to use CaEDTA systemically and EDTA locally. It was previously suggested (1) that CaEDTA protects against the toxin because Zn^{++}, Co^{++} and Mn^{++}, either of which are necessary for the action of the toxin in the presence of CaEDTA, are preferentially complexed over Ca^{++}. If this hypothesis obtains, the protection afforded by the CaEDTA when administered systemically suggests that the "free" metallic ions in the body that are preferentially complexed over Ca^{++} are complexed and prevented from playing an active role in the body. Inasmuch as these ions may play a role in the growth of some microorganisms or the activity of the toxins produced by them, the use of CaEDTA and related chelating agents may be of value in other infectious diseases. Also, inasmuch as the activity of some drugs, such as salicylic acid(2) and isoniazid(3), is thought to involve chelation, other chelating agents such as CaEDTA may exert a synergistic action by controlling the amount of "free" metallic ions in the body.

Summary. It has been demonstrated that CaEDTA can protect mice against a lethal dose of *Cl. perfringens* toxin, and guinea pigs

against experimental gas gangrene.

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Responses in Molt and Lay of Fowl to Progestins and Gonadotrophins.* (22588)

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The effects of progesterone in initiating proliferation of the resting feather papilla with consequent molt of the old plumage is well established(1), but there is as yet no clear picture of the point of attack of the steroid in this process. Local stimulation is entirely conceivable in view of the importance of the mesodermal component in feather regeneration. The experimental demonstration, however, has not proven entirely satisfactory(2), although the technics employed may later be found to have been inadequate. Alternatively, progesterone could be thought to act through its effects in the pituitary, the interruption of lay which follows its administration being especially suggestive in this regard. These alternatives were further investigated. Laying hens were treated with compounds other than progesterone having expected progestational activity in one series of experiments. Complementary experiments on possible interference of gonadotrophins or prolactin with the known response to progesterone were carried out simultaneously.

Methods. The hens used belonged to a strain of New Hampshires maintained at the University of Maryland Poultry Farm. They were in their first year of lay and had completed the normal autumnal molt when brought into the laboratory. Each bird was placed in a single cage and individual egg records were kept for over a month prior to treatment. Distribution of the birds to the experimental groups was made on the basis of these records. Individual egg records were continued during the period of treatment and for about 6 weeks thereafter. For tabular representation, weekly production per group was used. Date of molt was arbitrarily set as the day when all birds in the group had commenced a regular daily shedding of body plumage. Duration of molt was counted to the day when the entire group had ceased daily feather loss. No attempt was made to record feather number per hen but notations were made of noticeable differences in individual intensities of shedding. One control group served for all the concurrent experimental series. Testosterone propionate (TP), 50 mg/ml and desoxycorticosterone acetate (DCA), 10 mg/ml were dissolved in 8 parts sesame oil and 2 parts benzyl alcohol. Cortisone acetate (CA) was suspended in the same vehicle, 10 mg/ml. Progesterone (Pg.) was administered as a preparation having slow absorption from local depots.† Pregnant mares' serum (PMS) was Cutter's "Serum Gonadin." Follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (LTH) were Armour's‡ preparations.

Results. Series I. TP and DCA were injected as multiples of the Pg. dosage, corresponding roughly to the respective ratios given by Hooker and Forbes(3) for systemic administration in the mouse. Cortisone acetate was injected as the weight equivalent of the DCA dosage. Details of administration

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† Repositol Progesterone, Pitman-Moore Co.

‡ The FSH, LH and LTH preparations were most kindly furnished by Dr. I. M. Bunding, Armour and Co., Chicago, Ill.