THE PROPHYLAXIS OF EXPERIMENTAL ANTHRAX INFECTION WITH VARIOUS HORMONE PREPARATIONS.*

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Of the two factors involved in infectious disease—the host and the infecting agent—the latter has received the greater amount of laboratory study, both from the standpoint of those characteristic reactions which make recognition on isolation an easy task and its preparation for purposes of prophylaxis and therapy. It is only recently that attention has been turned to a study of the various factors, other than serologically demonstrable characteristics, which determine to some degree the ability of the host to resist infection or to overcome it once it becomes established, or which make one individual more susceptible than another to bacterial or virus invasion. Thus, it has been shown that such non-specific conditions as the nutritive state, climatic conditions, and physical constitution play more than a minor rôle in determining the resistance of man, not only to a specific type of infection but to parasitism by microbial agents in general.

Although it has been postulated by many that the endocrine system must play an important part in determining the degree of susceptibility or resistance to infectious disease, this has been done largely on the basis of inference drawn from observations made in the clinic, where it has often been noticed that individuals suffering with disturbance of function of one or another of the endocrine glands seem more prone to infection and do not overcome infectious processes with the same degree of ease as do those who have no apparent endocrine dysfunction. The experimental evidence in support of such an hypothesis is meager and contradictory. Search of the literature reveals many reports on the use of various hormone preparations in both natural and experimental infections, but no one disease has been thoroughly investigated from the endocrine standpoint, although a number ranging in severity from acne to tuberculosis, have been rather superficially studied. On the basis

of the evidence available, no conclusions can be drawn with regard to either the rôle that the endocrine system plays in determining resistance to susceptibility to natural infection or to the value of hormones, in general, in the prophylaxis and therapy of either experimental or natural infectious disease.

The hormone which has received the greatest attention in connection with infection is the one present in the cortex of the adrenal, since it has been noted that adrenalectomy reduces markedly the resistance of animals to various types of intoxicants. The literature contains many reports dealing with the employment of adrenal cortical extracts of various types in many experimental infections, but no definite conclusions can be drawn as to the efficacy of its use, since the evidence is contradictory. It is impossible to cite here all of the previous work on this subject; thus mention is made of but a few outstanding studies. Scott[24] was the first to demonstrate that adrenal cortex extracts exerted a protective effect against intoxication with both staphylococci and streptococci. The resistance of adrenalectomized rats to intoxication with killed *E. typhi* and *Staphylococcus aureus* was markedly increased by treatment with cortical substance (Hartman and Scott[8]). That this hormone is not always protective was shown by Scott, Bradford, Hartman, and McCoy,[25] who found no difference in the resistance of rats and guinea-pigs treated with it and of untreated controls infected with *Trypanosoma equiperdum* or injected with diphtheria toxin. The important function of the adrenal cortex in affecting susceptibility to bacterial intoxication has been demonstrated by Jaffe and Marine,[10] Jaffe,[11] and Marmorston-Gottesman and Perla,[19] who made the interesting observation that the decrease in resistance following adrenalectomy is not related to the mechanism of specific acquired immunity. This does not disappear after operation. Pottenger and Pottenger[22] have recently reported beneficial results in the treatment of tuberculous guinea-pigs with cortical extract.

No attempt will be made to review even partially the literature dealing with the use of adrenal cortical preparations in human infection. It will suffice to say that diseases ranging in severity from the common cold to typhoid fever and *Staphylococcus aureus* septicemia have been treated, with results permitting no definite conclusions.

The clinical observation that resistance to disease may vary with reference to the menstrual cycle and in pregnancy has led to the

investigation of the sex hormones from the standpoint of their use in experimental infections of various types. The outstanding work on this subject is probably that of Jungeblut and Engle,[12, 13, 14, 15] who, working on the hypothesis that the frequency of poliomyelitis in children and the rarity of the disease in adults bears a relationship to the activity of the sex glands, were able to show that the sera of animals treated with anterior pituitary hormone, pregnancy urine, and pregnant mare serum inactivated poliomyelitis virus in vitro, but that these endocrine secretions could protect immature animals infected with the virus only in exceptional instances. It can be concluded from their work that the sex hormones, either when administered or when present in large amounts, as in pregnancy, exert a very definite effect on the ability of sera to neutralize the agent of infantile paralysis. However, Hudson, Lennette, and King[9] were unable to repeat the work of Jungeblut and Engle and could not demonstrate any neutralizing power for poliomyelitis virus in the sera of young female monkeys treated with anterior pituitary extracts. Aycock[3] has, however, reported the protection of 5 of 6 castrated monkeys against poliomyelitis infection by treatment with estrogenic hormone.

Some attention has been focused on the relation of the sex hormones to experimental syphilis because of the clinical observation that invasion occurring simultaneously with or following conception in man frequently alters the course of the disease. Brown and Pearce[4] made the first experimental study of this subject and found that the early course of syphilis in a group of male and non-pregnant female rabbits was appreciably more severe than in a group of females in which impregnation and infection had occurred simultaneously, while Chesney[5] demonstrated that syphilis in the non-pregnant female rabbit is milder than in the male. Using a dose of 20 to 60 units of estrogenic hormone, Frazier, Mu, and Hu[7] administered the material subcutaneously for 15 days prior to intratesticular inoculation with *T. pallidum*, and, continuing the treatment for 250 days, found that the incubation period of the initial orchitis in rabbits was not influenced by treatment, but resolution of the testicular syphiloma occurred in about one-third the time in the treated as compared with the non-treated control animals. None of the experimental animals developed a metastatic orchitis such as occurred in about 70 per cent of the controls in whom generalized lesions

were three times more frequent than in the experimental group. In later work, Kemp[16] injected rabbits with estrogenic hormone and pregnancy urine factor (167 I.U. estrin + 80 rat units anterior pituitary-like hormone 6 days per week for 2 weeks and half of this amount per day for 13 additional weeks), infected them with *T. pallidum* 3 days following the beginning of hormone administration and found the disease in the treated animals to be as severe as in the untreated, in some instances there being a strong suggestion that the infection had been aggravated by treatment. The female rabbits fared better than the males, regardless of whether or not they were given gland products.

Magara,[17, 18] working with both male and female sex hormones, demonstrated a selective protective effect against bacterial invasion and intoxication, the male substance protecting only male and the female only female animals, both against infection with pneumococci (mice) and intoxication by tetanus and diphtheria toxins (guinea-pigs). In the latter experiment Magara found that, while the follicular hormone protected female animals against both types of toxin, the male substance protected males only feebly against diphtheria and was inactive against tetanus toxin. A study of the mechanism involved in the protection led the author to conclude that the increase in resistance was a general one and not due to a rise in the concentration of antitoxic substances in the sera of the treated group. Studies of the effect of the estrogenic and gonadotropic hormones on vaccinia and the Duran-Reynals spreading factor led Sprunt, McDearman, and Raper[26] to conclude that the administration of estrin had an effect on the spread of India ink and on the resistance of rabbits to vaccinia. It was found that this endocrine product, when given for one week, slows up the passage of the ink into the tissues, but allows it to reach and exceed a normal spread after 24 hours, whereas treatment for 3 weeks prior to injection gives a much smaller area of spread in the experimental than in the control animals. The resistance to vaccinia was found to be increased if rabbits were castrated and then given estrogenic hormone for a period of 3 weeks before being vaccinated.

Several studies have been made of the effect of the sex hormones on the course of experimental tuberculosis in guinea-pigs. Addessi[1] found that the disease was accelerated in animals treated with the whole urine of pregnant women, while Vercesi and Merenda,[28]

using isolated preparations of gonadotropic and estrogenic hormones, concluded that these do not aggravate tuberculosis in the experimental host. No effect could be demonstrated by Repetti[23] from the administration of the estrogenic substance, folliculin, on this infection in guinea-pigs. One of the most recent investigations on this subject is that of Steinbach and Klein,[27] who found that the experimental disease in rabbits and guinea-pigs was favorably influenced by the administration of Antuitrin-S, pregnant mare serum, and, to a lesser extent, follutein. No retardation of the tuberculous process was produced by the use of either anterior pituitary extract or emmenin.

Both Perla[20, 21] and Culbertson and Molomut[6] have shown that in experimental animals the removal of the hypophysis is associated with a drop in the resistance to both spontaneous bacterial and induced protozoan infection. The latter investigators were not able to increase the resistance of animals by administering pituitary extracts, while Perla, by injecting the operated rats with an alkaline extract of the anterior lobe of the pituitary, restored the resistance against *Trypanosoma lewisi* to normal levels.

The clinical observation that diabetic individuals are more prone to invasion by infectious agents and react more severely when infected than do normal people has stimulated a study of the effect of insulin on various types of experimental and natural infections in both diabetic and non-diabetic subjects. Although the number of diseases in which treatment with insulin has been attempted is rather large, attention has been focused largely on the use of this hormone in tuberculosis in non-diabetic human beings. No attempt will be made to review the extensive literature on this subject, this having been done by Allen.[2] It is enough to say that, at present, there is no general agreement as to the efficacy of insulin in the treatment of tuberculosis.

Various other hormones have been studied in both experimental and natural infections as prophylactic and therapeutic agents, with more or less indefinite results. The number of investigations carried out with any one substance is limited and, while beneficial results have been reported, no conclusions can be drawn with regard to their efficacy.

The purpose of the work reported here was to study the effect on a single experimental infection of as many potent hormone prep-

arations as could be obtained.   The investigation was designed to determine the prophylactic rather than the therapeutic properties of the various products.   The infection studied was anthrax; the experimental animals, mice.

MATERIALS AND METHODS

Male and female hybrid white mice, the animals used in the studies reported here, were kept in the laboratory for at least one week before any treatment was given and were fed a diet of oats and compressed food, containing all of the essential nutritive elements, throughout the experiments.

Sixteen different glandular preparations were used and injected subcutaneously in adequate amounts, the course of therapy covering, unless otherwise stated, 15 days (3 periods of 5 days each), one day of rest being interposed after every 5th day, and the infecting agent injected on the day following the last treatment.   The hormones were not administered after infection was established.   The following table lists the types of hormone used, their source, and the dosage given daily in most of the experiments here reported.   In some instances the amounts given varied from those described below, such differences being indicated in the sections dealing with the experiments in which they were used.

> Insulin protamine zinc (Lilly)—Males—0.025 U.   Females—0.05 U.
> Pineal extract (Rowntree)—0.50 cc.
> Thymus extract (Rowntree)—0.50 cc.
> Testosterone proprionate (Schering, and Dr. J. B. Hamilton, Department of Anatomy, Yale University School of Medicine)—0.25 mg.
> Progesterone (Schering)—0.10 mg.
> Thyroxine (Squibb)—0.01 mg.
> Beta-hypophamine (Parke-Davis)—0.1 cc.
> Alpha-hypophamine (Parke-Davis)—0.1 cc.
> Parathyroid extract (Parke-Davis)—2.5 Hanson Units.
> Gonadotropic hormone (Winthrop)—10 Rat Units.
> Extract of the whole anterior pituitary (Parke-Davis)—0.1 cc.
> Adrenal cortical extract (Upjohn)—0.5 Rat Unit.
> Estrin (Parke-Davis)—20 I.U.
> Thyrotropic hormone (Parke-Davis)—0.1 cc.
> Growth hormone of the pituitary (Parke-Davis)—1 Growth Unit.
> Prolactin (Ayerst, McKenna and Harrison, and Dr. Abraham White, Department of Physiological Chemistry, Yale University School of Medicine)—5 Rat Units.

Although the ideal control materials for use in these experiments are inactivated preparations of the various protein-containing hormones, such are not permissible since there is no agreement as to the best procedures for destroying all of the activity of these substances.   Animals which served as controls were injected, therefore, with 0.1 cc. of a 1 per cent peptone solution in order to control the non-specific protein effect encountered by the use of any hormone preparation containing an appreciable amount of protein material. While the peptone solution was employed in most of the experiments, several tests were carried out using a 2 per cent solution of crystalline egg albumin (0.1 cc. constituted the daily dose) in order to eliminate the size of the protein molecule as a factor in any effect which might be observed, since the protein hormones probably consist of molecules considerably larger than those found in peptone. Since the sex hormones (estrin, progesterone, and testosterone) were administered in solutions of sesame oil, 0.1 cc. of this substance containing no hormone was administered to another group of animals which acted as controls on those receiving the oil-soluble substances (0.1 cc.).

A 24-hour culture of *B. anthracis* grown on heart-infusion agar at 37° C. was employed as the infecting agent in all of the experiments here reported.   Determinations of the M.L.D. were made and at least 100 times this amount was given in a volume of 0.1 cc. of physiological saline as the infecting dose; in some experiments 1000 to 10,000 M.L.D. were given, this fact being mentioned in the sections describing these tests.   The organisms were always inoculated subcutaneously in a region removed from the point of injection of the hormone in order to eliminate any direct effect which might result from contact between bacteria and any residual endocrine material which had not yet been absorbed.   This precaution was found especially necessary in animals receiving oil-hormone solutions, since oil cysts were formed in almost every instance at the site of injection.
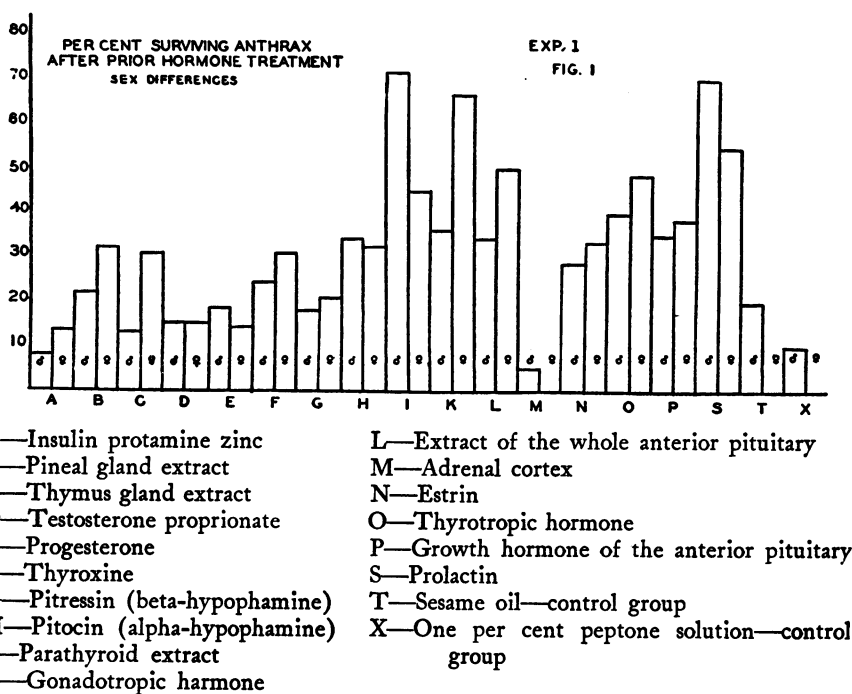
The number of animals dying in each 24-hour period following injection of the bacteria was noted, and all experiments were continued for 216 hours following infection.   Many of the mice in the various treated and untreated groups which died were autopsied, and smears were made of the heart blood and spleen in order to detect the presence of anthrax bacilli.   In some instances cultures

were made of the organs and the recovered organisms identified by means of cultural and pathogenicity tests.

RESULTS

Effect of Prior Treatment with Various Hormones on Resistance to Anthrax Infection

Experiment I. Using groups of male and female mice varying in number from 17 to 25, each of the 16 gland preparations was injected subcutaneously for the specified period of time and the animals infected with *B. anthracis.* Figure 1 illustrates the num-

PER CENT SURVIVING ANTHRAX AFTER PRIOR HORMONE TREATMENT SEX DIFFERENCES   EXP. 1   FIG. 1

A—Insulin protamine zinc
B—Pineal gland extract
C—Thymus gland extract
D—Testosterone proprionate
E—Progesterone
F—Thyroxine
G—Pitressin (beta-hypophamine)
H—Pitocin (alpha-hypophamine)
I—Parathyroid extract
K—Gonadotropic harmone

L—Extract of the whole anterior pituitary
M—Adrenal cortex
N—Estrin
O—Thyrotropic hormone
P—Growth hormone of the anterior pituitary
S—Prolactin
T—Sesame oil—control group
X—One per cent peptone solution—control group

ber of survivors, 216 hours following infection, in the various treated and control groups, the results being presented according to sex. It will be noted that while, with some of the endocrine substances, some differences were detectable in the number of animals resistant to invasion according to sex, these were not very large and in most instances the number of animals not succumbing to the infection when treated with any one hormone showed little or no sex varia-

tion. It is interesting that the male and female hormones (testosterone and estrin) produced no selective effect, both sexes reacting in the same manner to the injection of either one. These results are in direct contradiction to those of Magara[17] who found the female sex hormone capable of protecting only female and the male substances only male mice against pneumococcus infection.

Figure 2 presents the average percentage of survivors in the treated and control mice, according to treatment, grouping the male and female animals together. While none of the hormones, in the dosages used, were found to reduce appreciably the resistance of the treated animals, they did not all protect to the same degree, and they could be divided into three groups according to their effect in decreasing the susceptibility to anthrax infection. Thus, the substances which seemed to have little or no protective effect in this experiment were insulin, testosterone proprionate, progesterone, beta-hypophamine, and adrenal cortex. Although it is obvious that it is very difficult to
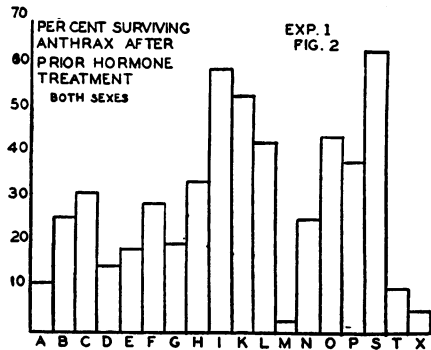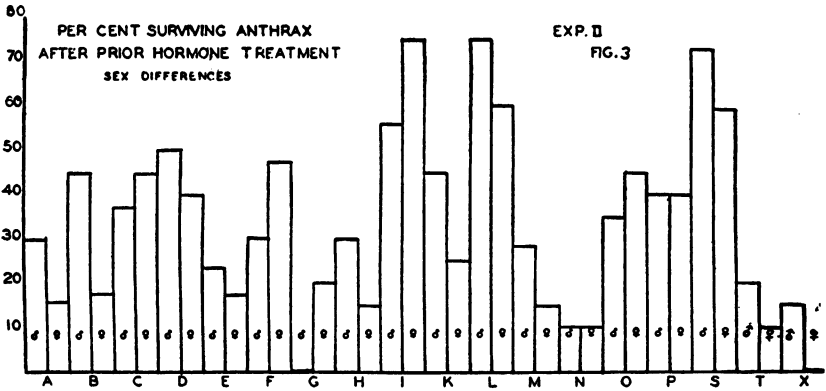

Legend as in Fig. 1

draw a hard and fast line between those substances showing a moderate degree of protection and those producing little or none, nevertheless an examination of the graph indicates that extracts of the pineal and thymus glands, thyroxine, alpha-hypophamine, estrin, and the growth hormone of the pituitary gland exerted a more marked protective action in general than did adrenal cortex, or the peptone or oil used in the controls. Difficulty also arises in separating accurately those substances producing the most marked protective effect from those in the upper levels of the moderately active group, but it is apparent that prolactin, parathyroid extract, gonadotropic hormone, extract of the whole anterior pituitary, and thyrotropic hormone exerted the greatest degree of protection when administered prophylactically.
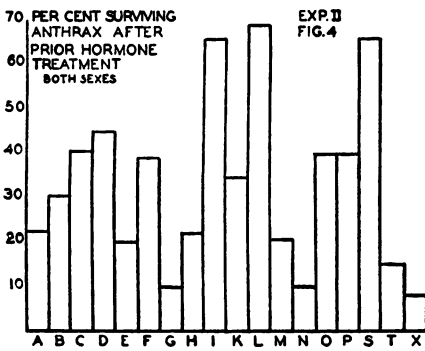
Experiment II. This is a duplicate of Experiment I. It was performed some time later and involved the use of the same hormones in groups of male and female mice ranging in number

from 17 to 25. It will be noted from Figure 3 that, as in the previous experiment, little sex difference could be detected in the results obtained with any one hormone. Again, no differences could be

Legend as in Fig. 1.

found in the reaction of male and female mice to the respective sex hormones. Figure 4 presents graphically the average percentage of survivors in the treated and control animals when the sexes were grouped together. In this experiment the hormones again can be divided into three groups on the basis of the degree of protection produced. While the substances which in this experiment showed little or only moderate increase of resistance are not all the same as those found in the previous tests (Experiment I), this is not unusual

Legend as in Fig. 1.

in view of the difficulties involved in experiments of this type. Differences in potency of preparations of the same hormone used at different times must be taken into consideration, since it is an established fact that many of the endocrine substances tend to lose potency on being stored, and that two different preparations of the same substance made at different times may show variable activity. Taking this into consideration, together with the fact that studies of infection in animals introduce many variable factors which cannot

be controlled, the results obtained in this experiment are sufficiently close duplications of the data obtained previously to make them valid. The endocrine substances which exerted marked protective effect in the first experiment endowed the animals with appreciably increased resistance when used the second time. Thus, prolactin, extract of the whole anterior pituitary gland, and parathyroid extract treatment reduced the mortality of the treated groups to a point where over 65 per cent of these mice survived infection, whereas only 10 to 15 per cent of the control animals were living at the termination of the experiment.

Summary of Experiments I and II. The average percentage of survivors in both experiments, grouped together regardless of sex, (68 to 100 mice in each group) are presented in Figure 5. It can be seen that the endocrine sub-stances tested fall more or less into three groups, even when the results of the two tests are aver-aged. Those which exhibited very little or no protective effect included insulin, progesterone, estrin, extract of the adrenal cor-tex, and beta-hypophamine, while 5 of the hormones examined fell into the group which exerted a moderate effect. These were ex-tract of the pineal body, extract
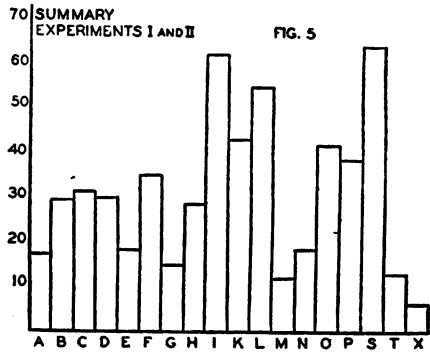
Legend as in Fig. 1.

of the thymus gland, thyroxine, alpha-hypophamine, and testosterone proprionate. These substances yield more or less irregular effects as evidenced by the data given above (Expts. I and II) in which some protected at one time to a much higher degree than at another. Thus, the use of testosterone proprionate in Experiment I led to the survival of only 15 per cent of the animals, while in the second experiment the same treatment preserved the lives of 45 per cent of the mice. The hormones which showed the highest protective effect when given prophylactically were found to be extract of the parathyroid gland, the growth hormone of the pituitary, thyrotropic hormone, prolactin, gonadotropic substance, and extract of the whole anterior pituitary body. Examination of the graph shows definitely that the degree of protection exerted by these materials was very

high, even when the results of both experiments were averaged, two of them saving from 60 to 65 per cent of the infected animals, while the other caused the survival of from 40 to 55 per cent. These figures may be compared with the 6 to 12 per cent of the controls surviving.

Interesting results are obtained when the various hormones are classified according to source. Most of the sex hormones, excluding the one from the pituitary gland (gonadotropic), proved to be of little value in increasing the resistance to anthrax infection, the exception being testosterone proprionate which produced rather irregular results but which, on the basis of averaging the data obtained in several tests, could be included in the group of endocrine materials affording a moderate protective effect. Of the substances obtained from the posterior portion of the pituitary, alpha- and beta-hypophamine, only the former showed any ability to increase the resistance. It is most interesting that the hormones present in the anterior pituitary possessed the resistance-increasing power to the highest degree. Thus, gonadotropic, growth, thyrotropic, lactogenic hormones, and extract of the anterior pituitary, containing probably small amounts of several active substances, were found to give the best results. The remaining endocrine materials, which comprise a miscellaneous group, were two hormones which exerted no effect (adrenal cortex and insulin), several which were able to decrease susceptibility but moderately (extracts of the thymus and pineal glands, and thyroxine), and one (extract of the parathyroid) which increased resistance to the same degree as the most potent of those derived from the anterior pituitary.

Two experiments, independent of the tests described above, were performed to the end of determining whether or not the size of the protein molecule had any effect on the susceptibility to infection. Two groups of female mice were used in each experiment, one receiving 0.1 cc. of anterior pituitary extract for the prescribed length of time, the other being injected daily with 2 mg. of crystalline egg albumin. Infection with anthrax in the first experiment, using 1000 M.L.D., showed that, whereas 60 per cent of the animals receiving the pure protein succumbed in 24 hours, and 100 per cent in 48 hours, none of the hormone-treated mice were dead until 48 hours following injection, and 60 per cent still survived 216 hours after inoculation with the organisms. The second experiment, in which 10,000 times the minimum infective dose was used

to inoculate the animals, showed practically the same results as the first, except that only 40 per cent of the treated group were still alive at the conclusion of the experiment.   It is obvious from the above data that the size of the protein molecule plays no rôle in the protection which some of the hormones were found to exert and that the injection of 1 per cent peptone solution serves as an adequate control procedure.

Death Rates of Animals with Anthrax Infection after Prior Treatment with Hormones

The number of animals succumbing to anthrax infection in each 24-hour period following infection was noted in order to determine the rate of death, with a view to ascertaining whether or not the treated animals showed a slower rate of dying at the beginning of the experiment than did the controls and if this speed was constant or increased as time elapsed.   The data obtained in Experiments I and II are presented in Tables 1 and 2.   It will be noted that in practically every instance the death rate corresponded very closely to the degree of protection as evidenced by the number of survivors at the end of the experiments.   Thus, the animals which received the control solutions and the ineffective hormone showed the steepest, those to whom moderately protective endocrine materials were administered moderate, and the mice injected with the most potent resistance-increasing hormones the flattest slopes in their death-rate curves.   The rates for several groups were plotted logarithmically to determine the constancy of the speed with which they succumbed and a straight line was obtained; evidence that the deaths were occurring in each group at a constant rate.   This graph is not included here.   Figure 6, based on the data obtained in Experiment II, illustrates graphically the death-rate curves of several groups of mice treated with one hormone which exerted no effect, with one which had a moderate influence, and with two which were highly protective.   The relation of the slope of the death-rate curve to the percentage survival is apparent.   It is most interesting to note, also, that in the case of prolactin, none of the animals were dead until 72 hours had elapsed after infection, while with extract of the anterior pituitary this latent period was extended to 120 hours. These results seem very significant since they indicate that, although a certain number treated with these hormones eventually died, the mice were endowed with a sufficient degree of resistance to with-

TABLE 1.

DEATH RATES OF ANIMALS RECEIVING TREATMENT WITH VARIOUS HORMONES PRIOR TO INFECTION WITH *B. anthracis*

Experiment I.

Per Cent Dead at Various Hours

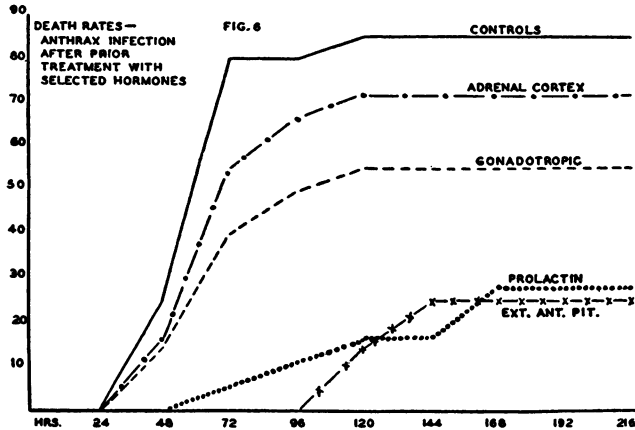| Treatment	Sex	24	48	72	96	120	144	168	192	216
Sesame oil	♂	0	29	57	74	80	80	80	80	80
	♀	0	23	70	93	100	100	100	100	100
Peptone water	♂	0	40	60	80	80	90	90	90	90
	♀	0	8	70	84	91	100	100	100	100
Progesterone	♂	0	9	45	55	73	82	82	82	82
	♀	0	20	52	73	86	86	86	86	86
Estrin	♂	0	11	33	60	71	77	82	82	82
	♀	0	27	55	67	67	67	67	67	67
Testosterone	♂	0	14	50	85	85	85	85	85	85
	♀	0	20	33	53	59	66	85	85	85
Gonadotropic	♂	0	0	18	45	64	64	64	64	64
	♀	0	6	16	33	33	33	33	33	33
Adrenal cortex	♂	0	25	50	75	75	85	95	95	95
	♀	0	23	70	81	81	100	100	100	100
Pitressin	♂	0	16	58	75	75	82	82	82	82
	♀	0	16	66	71	71	75	79	79	79
Pitocin	♂	0	6	17	39	39	39	55	66	66
	♀	0	27	56	68	68	68	68	68	68
Parathormone	♂	0	7	21	28	28	28	28	28	28
	♀	0	10	20	35	50	55	55	55	55
Thyroxine	♂	0	17	52	58	58	65	65	76	76
	♀	0	15	61	69	69	69	69	69	69
Thymus extract	♂	0	43	68	80	87	87	87	87	87
	♀	0	27	55	55	61	61	61	69	69
Pineal extract	♂	0	26	40	46	52	65	78	78	78
	♀	0	15	30	40	50	57	68	68	68
Insulin (Protamine)	♂	0	15	69	77	85	92	92	92	92
	♀	0	31	56	68	81	87	87	87	87
Prolactin	♂	0	10	20	30	30	30	30	30	30
	♀	0	20	25	40	45	45	45	45	45
Growth hormone	♂	0	15	30	55	65	65	65	65	65
	♀	0	20	37	56	62	62	62	62	62
Anterior pituitary	♂	0	20	50	66	66	66	66	66	66
	♀	0	22	33	44	50	50	50	50	50
Thyrotropic	♂	0	30	50	60	60	60	60	60	60
	♀	0	13	39	52	52	52	52	52	52

TABLE 2.

DEATH RATES OF ANIMALS RECEIVING TREATMENT WITH VARIOUS HORMONES PRIOR TO INFECTION WITH *B. anthracis.*

Experiment II

Per Cent Dead at Various Hours

Treatment	Sex	24	48	72	96	120	144	168	192	216
Sesame oil	♂	0	5	40	75	80	80	80	80	80
	♀	0	15	45	80	90	90	90	90	90
Peptone water	♂	0	25	80	80	85	85	85	85	85
	♀	0	20	70	100	100	100	100	100	100
Progesterone	♂	0	6	59	71	71	71	77	77	77
	♀	0	17	44	72	83	83	83	83	83
Estrin	♂	0	15	80	90	90	90	90	90	90
	♀	0	15	60	80	85	85	90	90	90
Testosterone	♂	0	5	30	45	50	50	50	50	50
	♀	0	10	25	50	55	55	60	60	60
Gonadotropic	♂	0	15	40	50	55	55	55	55	55
	♀	0	15	35	50	60	65	70	75	75
Adrenal cortex	♂	0	17	55	66	72	72	72	72	72
	♀	0	10	70	80	80	80	85	85	85
Pitressin	♂	0	27	81	92	100	100	100	100	100
	♀	0	20	60	70	80	80	80	80	80
Pitocin	♂	0	25	55	60	60	60	70	70	70
	♀	0	25	70	80	80	85	85	85	85
Parathormone	♂	0	6	17	44	44	44	44	44	44
	♀	0	0	10	15	20	25	25	25	25
Thyroxine	♂	0	45	70	70	70	70	70	70	70
	♀	0	18	47	53	53	53	53	53	53
Thymus extract	♂	0	6	19	44	57	63	63	63	63
	♀	0	11	22	33	44	55	55	55	55
Pineal extract	♂	0	10	50	55	55	55	55	55	55
	♀	0	11	44	66	72	83	83	83	83
Insulin (Protamine)	♂	0	30	50	60	60	60	70	70	70
	♀	0	15	69	77	84	84	84	84	84
Prolactin	♂	0	0	6	11	17	17	28	28	28
	♀	0	5	15	25	40	40	40	40	40
Growth hormone	♂	0	20	40	50	55	55	60	60	60
	♀	0	5	30	40	50	50	60	60	60
Anterior pituitary	♂	0	0	0	0	15	25	25	25	25
	♀	0	15	30	35	35	35	40	40	40
Thyrotropic	♂	0	15	25	45	50	50	65	65	65
	♀	0	10	45	50	55	55	55	55	55

stand the infection for a much longer period than did the control animals, 80 per cent of which had succumbed 72 hours after injection of the bacteria. Thus, while complete protection was not pro-


duced in every animal in the groups treated with prolactin and anterior pituitary, all of the mice had some degree of increased resistance, the least protected dying at a much later time than the controls.

Effect of Prior Treatment with Combinations of Two Hormones on the Resistance to Anthrax Infection

Certain reported work indicates that two or more hormones may act in a synergistic manner in evoking a physiologic reaction. It seemed that the same type of response might extend to the protective effect of the hormones. Consequently, several groups of female mice were given simultaneous injections of several combinations of two hormones, each being injected separately in the same dosage as when used alone. The period of treatment was the same as that used for the single hormones. At the same time that the combinations were being given, other groups of female mice were injected with each of the hormones separately, these groups serving as controls. The results are presented in Figure 7.

It is apparent that not only was a synergism not demonstrable, but that in most instances the activity of the more potent hormone of the combination was reduced when it was given in conjunction with another potent substance. In only a single instance was such

a reduction not observed, and in this case the effect was no greater than that produced by one hormone alone. The reason for this reduction in protective effect is not apparent at the moment; it seems strange that even though no synergistic effect resulted from the combination of two resistance-increasing materials, the activity of
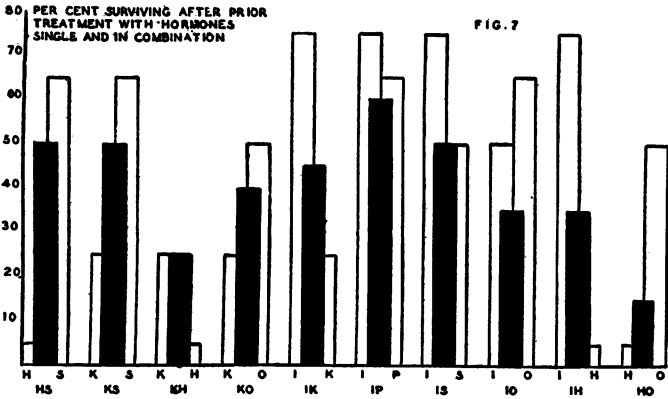
Legend as in Fig. 1. Unshaded—Survivors following treatment with a single hormone. Shaded—Survivors following treatment with combination of 2 hormones.

the more potent component would not be exhibited. Further study of this phase of the problem is clearly indicated.

Effect of Prior Injection of Varying Doses of the Sex Hormones and Thyroxine on Resistance to Anthrax Infection

The sex hormones, estrin, testosterone proprionate, and gonadotropic substance, and also thyroxine, were administered to groups of from 20 to 25 mice, male and female, in varying doses. Treatments were given daily for one week, followed by one day of rest, with treatment then repeated for another week, infection taking place on the day after the last injection. Only one dose of thyroxine (0.01 mg.) was used, since it was found that amounts larger than this were not tolerated, death, without infection, occurring in a large percentage of the animals after about 8 to 10 days of treatment. Both high and low doses of the other substances were administered; of estrin 100 I.U. and 20 I.U., of testosterone 1.25 and 0.12 mg., and of gonadotropic hormone 1 and 10 rat units. Two separate experiments were carried out at different times; with results as appear graphically in Figures 8 and 9.

It can be seen that there is very little difference attributable to

sex in the reaction to the several hormones.   Here again, the results
are in contradiction to the conclusions of Magara with regard to
a selective action of the sex hormones for the sex from which they
are derived.   It is obvious from these figures and from those of

EFFECT OF SELECTED HORMONES IN VARYING DOSES ON RESISTANCE TO ANTHRAX
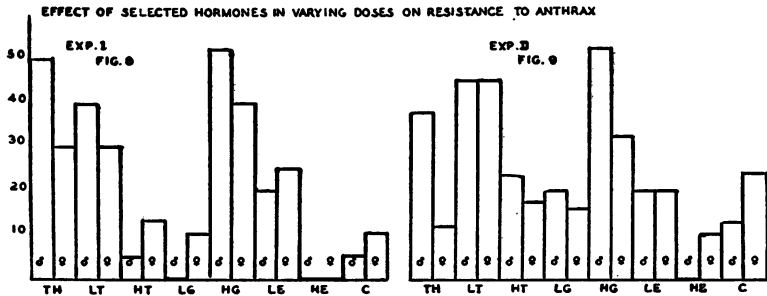
TH—0.01 mg. thyroxine	HT—1.25 mg. testosterone proprionate
LE—20 I.U. estrin	LG—1 Rat unit gonadotropic hormone
HE—100 I.U. estrin	HG—10 Rat units gonadotropic hormone
LT—0.12 mg. testosterone proprionate	C—0.1 cc. sesame oil—control group

Figure 10, which represents a summary of both experiments with-
out separating the data according to sex, that the amount of

SUMMARY
EXPTS I AND II   FIG. 10

Legend as in
Figs. 8 and 9.

endocrine material administered plays an impor-
tant rôle in determining the type of effect pro-
duced.   Thus, while small doses of both estrin
and testosterone proprionate were found to be
somewhat protective against anthrax infection,
large doses not only failed to increase resistance
but even reduced to some extent the percentage of
mice surviving infection as compared with the con-
trol group.   The opposite effect was observed,
however, with gonadotropic hormone; here only
the larger dose increased resistance.   This may
be due to the fact that the smaller quantity used
is not sufficient to produce any effect, whereas the amount designated
in these experiments as the large dose approached more closely a
physiologically active quantity.   Considerably higher doses of this
hormone may be required before the moderate increase in resistance
with which it was found to endow experimental animals can be
overcome.

The reason for the harmful effect of large doses of testosterone
and of estrin is, of course, not known, but it may be suspected that
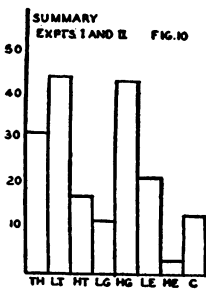
these hormones, like other pharmacologically active substances, exert a beneficial action only in optimum concentrations, and when given in much larger amounts may act as poisons.   Whether or not this interpretation is valid for all of the hormones used in this work remains to be determined.   That thyroxine is harmful in larger doses than those here used has already been mentioned; the margin is narrow between that dose required to exert moderate protection against infection and that capable of producing a state of thyrotoxicosis.   However, the results obtained with thyroxine in these experiments confirm the findings made in earlier work to the effect that this material may exert a moderate prophylactic effect against anthrax infection in mice.

Effect of Prior Treatment with Single Doses of 1000 I.U. of Estrin on Anthrax Infection

Since a single adequate dose of estrogenic hormone will evoke a physiologic response—cornification of the vaginal mucosa within a short time after its administration,—several experiments were per-
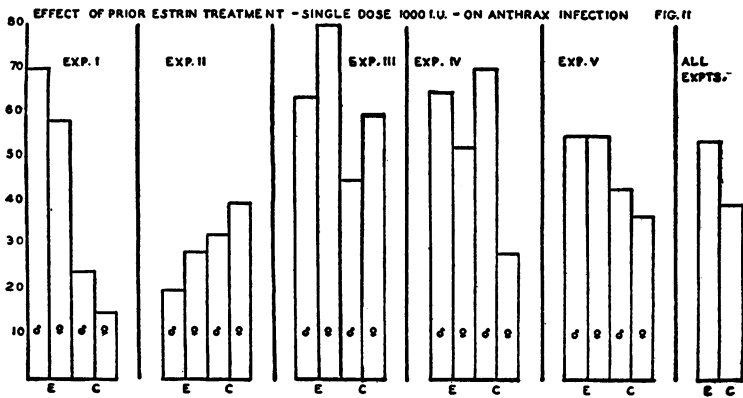
E—1000 I.U. estrin in one dose of 0.1 cc. volume in oil
C—0.1 cc. sesame oil—control group

formed to determine whether this type of treatment altered resistance to anthrax.   Groups of about 25 male and female mice were injected with a single dose of estrin, 1000 I.U., vaginal smears examined, and inoculations of an adequate dose of B. anthracis were given when the mucosa presented the appearance characteristic of full estrus.   The males were all infected 3 days after treatment. Control animals received sesame oil prior to infection.   Five sepa-

rate experiments following this procedure were performed at different times.   The results presented in Figure 11, together with the summary of the whole series of tests (graph at the right of Figure 11) show quite clearly that the administration of a single dose of estrin, although a large one, had very little effect on the susceptibility to bacterial invasion.   While in several of the experiments there was some evidence of protection, in others the hormone decreased resistance.   These findings bear out the contention that the endocrine substances require an adequate period of time for their action, prior to infection, if a protective effect is to be observed. When estrin was administered in smaller doses daily for more than 2 weeks, as in the experiments previously described, a somewhat greater increase in resistance was noted, although even then, this hormone was not found to be highly effective.

SUMMARY AND DISCUSSION

The data offered above seem to indicate that there are several hormones which possess the ability to increase the resistance of mice to invasion by anthrax bacilli, if treatment is instituted some time before the infecting injection.   On the basis of the results obtained, the endocrine substances can be divided into three more or less distinct groups according to the activity which they exhibit; one which shows little or no resistance-increasing power, another which decreases susceptibility but moderately, and a third which exerts a marked protective action when used as a prophylactic agent against anthrax.   It is interesting that, with the exception of the extract of the parathyroid gland, all of the other potent substances are present in the anterior portion of the pituitary body.   The sex hormones, to which significance has been attached in connection with their ability to ameliorate infection, were found to be relatively ineffective in this work, estrin and progesterone producing little or no effect, testosterone proprionate but a moderate degree of protection.

A study of the death rates of the various treated and control groups brings out clearly the fact that when protection occurred with some of the more effective hormones it was not necessarily complete in every animal.   This is shown by the fact that, although not all of the treated mice survived the infection, in some experiments none of those which died succumbed until an appreciable

time after deaths had appeared in the control groups; in one series, 80 per cent of the latter group having died before the first of the hormone-treated animals succumbed. This finding is important, because it emphasizes the fact that partial protection may result from treatment, and that the degree of immunity produced by the hormones is even greater than that shown by a mere tabulation of the number of survivors in the various control and test groups.

The question of adequate dosage arises in a consideration of the endocrine materials which exhibited little or no protective effect. While it has been shown that large quantities of some hormones— estrin and testosterone—lead to a decrease in resistance to infection, this fact has not been established for all of the non-protective substances. Adrenal cortical hormone, a rather small dose of which was used in the experiments here reported, should be studied further, utilizing larger amounts, since the reports in the literature on this material indicate that it tends to increase resistance to intoxication and to infection, and it is known, also, that adrenalectomy markedly decreases resistance. An operative procedure of this type introduces, however, a number of factors other than mere removal of the glands. These are difficult to control.

The results obtained in the administration of two hormones simultaneously are very puzzling, since it is difficult to explain why this procedure yielded a smaller number of survivors than did one of the substances when used alone. It is possible that the findings noted were due to the fact that the endocrine materials were not mixed in the proper proportions. That two or more hormones may be more effective than is a single one is indicated by the fact that potent extracts of the whole anterior pituitary body, which are known to contain activating factors for several endocrine glands, yielded very good results. Prolactin alone, however, exhibited as marked a degree of protection as did the whole anterior pituitary, but the effect of the latter cannot be attached necessarily to its prolactin content. The amount of lactogenic substance present in the pituitary preparation used was very small. Possibly the best effects can be produced when small amounts of several of the gland-activating substances in the anterior pituitary are used simultaneously. It must be kept in mind, however, that none of the anterior pituitary hormones used, such as prolactin, thyrotropic, growth, and gonadotropic, was a pure preparation; they probably consisted of a large quantity of the specific principle together with smaller quantities

of other of the pituitary hormones.   The findings obtained may be due, therefore, essentially to several substances acting in conjunction rather than to one alone.

No inferences can be drawn from these results in the prophylaxis against anthrax with reference to the activity of the effective hormones as preventives against other types of experimental infection. Although the method of inducing the disease in these studies approximates closely the natural process of infection, since the bacteria were introduced subcutaneously, no conclusions can be drawn as to the action of the hormones in naturally occurring disease.   Thus, although the results here reported are suggestive, other infections, both natural and experimental, must be studied before any broad general conclusions can be drawn as to the prophylactic value of different hormone preparations.   Also, no evidence is available to indicate that these potent endocrine substances possess therapeutic properties.   Wholly other conditions of infection and of treatment must be established for the study of this problem.

The mechanism involved in the production of the increased resistance by some of the hormones cannot be explained; indeed, these experiments were not designed toward that end.   Magara[18] has shown that, although the sex hormones decreased susceptibility to tetanus and diphtheria intoxications, no increase in antitoxins could be disclosed in the sera of such animals.   On the other hand, Weinstein[29] has recently presented evidence that the administration of large doses of estrogenic hormone produces an increase in the normal agglutinin (*E. coli*) and hemolysin (sheep cell) in rabbits. Since estrin has been found to be only slightly effective in increasing the resistance of mice to anthrax infection it would seem that any effect which this substance might have on serological reactions is not primarily concerned in altering the susceptibility to infection.

CONCLUSIONS

1.   The hormones present in the anterior pituitary gland— prolactin, thyrotropic hormone, gonadotropic hormone, the growth complex, and extract of the whole anterior pituitary—were found to be highly effective in protecting mice against infection with large doses of *B. anthracis* when these substances were given over a period prior to injection of the organisms and discontinued after the time of bacterial inoculation.   Parathyroid extract was the only sub-

stance not derived from the anterior pituitary which exerted a high degree of protection. Extracts of thymus and of pineal gland, thyroxine, testosterone proprionate, and alpha-hypophamine from the posterior portion of the pituitary were found to increase the resistance of mice to anthrax infection to but a moderate degree. Insulin, estrin, adrenal cortex, progesterone, and beta-hypophamine caused little or no change in susceptibility.

2. Studies of the death rates of treated and control animals showed that the number of survivors at the termination of an experiment could be correlated with the slope of the death-rate curve. Thus, in instances where a highly protective hormone was administered the treated animals did not start dying until some time after deaths appeared in the control groups or in those which had received substances which were only slightly or moderately effective. This indicates that although not all of the animals were protected to the point where they were able to overcome the infectious process and thus survive, all were endowed with some increase in resistance,—in many instances sufficient to delay death for an appreciable time.

3. No synergistic protective effect could be demonstrated by the simultaneous administration of two protective hormones. In almost every instance a decrease in activity of the more active preparation included in the combination was produced. No explanation for this effect is offered.

4. The quantity of the sex hormones administered was found to determine to a large degree the type of reaction produced. Thus, while small doses of estrin or of testosterone proprionate brought about a moderate increase in resistance in some experiments, large quantities of these not only failed to exert a protective effect, but, in some instances, decreased the ability of the animals to withstand infection to a point below that of the control groups. Gonadotropic hormone was found to be inactive when given in small doses; large amounts apparently increased resistance.

5. The administration of a single injection of 1000 I.U. of estrogenic hormone did not materially affect the ability of the treated animals to withstand infection with anthrax. The results obtained were very variable, and on the whole should be considered as essentially negative.

6. The mechanism of the protection induced by the effective endocrine preparations cannot be explained on the basis of the data

presented here.   It seems possible that the increase in resistance may not be related to serological changes.

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