

EFFECTS OF HORMONES ON THE ADRENAL NECROSIS
PRODUCED BY *BESNOITIA JELLISONI* IN GOLDEN
HAMSTERS*

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PLATES 13 TO 17

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Destruction of the adrenal gland that may lead to Addison's disease in man, has been traced to: *Mycobacterium tuberculosis* (13), *Histoplasma capsulatum* (19), *Cryptococcus neoformans* (19), *Coccidioides immitis* (17), and *Blastomyces* (27), whereas *Spirochaeta pallida* and viruses have been suspected as etiologic agents (12). The pathogenesis of adrenal necrosis produced by microorganisms has however remained obscure. It is noteworthy that the adrenal gland can be extensively parasitized, leading to almost complete destruction, whereas other organs of the same host may be affected slightly or not at all.

Parasitization and necrosis of the adrenal gland are only rarely seen in experimental infections. This is true for tuberculosis in guinea pigs, hamsters, and monkeys; however occasionally, acute generalized histoplasmosis in rabbits is associated with involvement of the adrenal gland. It appears that the only report of an actively progressive infection in the adrenal gland, unaccompanied by such lesions in other organs, is that from hamsters with chronic toxoplasmosis (6) (Fig. 7). Such animals had generally been infected for several months. They harbored *Toxoplasma* cysts in the brain and eyes, which gave rise to lesions only incidental to their rupture. Some of their organs appeared to contain also proliferative forms of *Toxoplasma*, which did not produce significant lesions and were demonstrable only by subinoculation. Rarely were functionally significant lesions produced in the eyes by small numbers of organisms. However, in the adrenals of such hamsters large numbers of *Toxoplasma* caused extensive destruction of the cortex, the medulla, or of both. Instances of such adrenal necrosis were found only inconstantly in animals that had been infected for several months; hence it was difficult to assemble sufficient animals for experimental studies.

During the study of another protozoan, *Besnoitia jellisoni*, which has recently been described (7, 10), adrenal necrosis was observed both during acute

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infection of hamsters as well as after several months, during chronic infection (8). Adrenal necrosis was present almost regularly at death from acute infection during the 2nd and 3rd week after subcutaneous inoculation, although generally, pneumonia was the immediate cause of death. About half of the hamsters that survived for several months died with bilateral adrenal necrosis and the clinical signs of weight loss, asthenia, diarrhea, and dehydration. At autopsy gross and microscopic lesions were prominent only in the adrenal glands (Figs. 1 to 3, 5 and 6, 8), in which they appeared directly attributable to *Besnoitia* organisms which were present intracellularly in large numbers (Figs. 9, 13). Ocular lesions were also frequently found (9). Other organs were either free of lesions, sometimes showing residuals of past necrosis, or they

TABLE I
Titration of *B. jellisoni* (P-181) in Hamsters
0.2 cc. was given subcutaneously

Dilution of peritoneal fluid	Day of death							Proportion with adrenal necrosis		
10^{-2}	9	9	9	10	10	10	10	10	11	7/10
10^{-3}	10	11	11	11	11	12	13			4/6
10^{-4}	13	13	15	16	D	S*				4/4
10^{-5}	14	17	S	S	S	S				2/2
10^{-6}	S	S	S	S	S	S				
10^{-7}	S	S	S	S	S	S				
LD ₅₀ = ID ₅₀ = $10^{-4.47}$ (20)										17/22

* S, survival.

contained a few small lesions accompanied by organisms so rare that they could be demonstrated better by subinoculation than in sections.

Chronic infection with *B. jellisoni* resembles certain cases of tuberculosis and histoplasmosis in that areas of adrenal necrosis may be the predominant or the only actively progressive sites of infection in the host, with other organs only minimally involved. Preliminary to a study of the pathogenesis of such late lesions, this paper presents an analysis of the factors influencing the development of adrenal necrosis during the acute infection.

A general review of the pathogenesis of *Besnoitia* infection in hamsters is required prior to the consideration of specific factors affecting the occurrence of adrenal necrosis (10, 11). *Besnoitia* is a protozoan multiplying only intracellularly by multiple binary fission. Inoculation of hamsters with either proliferative or cyst forms of *Besnoitia* leads to an acute infection in which necrosis is related to the parasitism of individual cells by proliferative forms. After subcutaneous inoculation, a generalized infection develops with lesions predominantly at the injection site, the regional and

distal lymph nodes, the lungs, liver, spleen, and the adrenal cortex. Untreated hamsters die with an acute infection between the 8th and 12th day (Table I). Prophylactic treatment of infected hamsters with sulfadiazine (Table II) may retard the proliferation of organisms sufficiently to prevent death. It also permits the development within 10 to 20 days of a state of relative immunity, resulting in survival after the drug is withdrawn. Graded sulfadiazine treatment was utilized in all the experiments to afford a longer period of observation and to study infection before, during, and after onset of immunity.

Surviving animals remain infected as can be shown by the subinoculation of their

TABLE II

Sulfadiazine Treatment of B. jellisoni (P-181) Infection in Hamsters

0.2 cc. of a 10^{-2} Dilution used as Subcutaneous Inoculum. Duration of Treatment: 16 days. Acute-effects observation period: 60 days.

Drug concentration	Unoperated			Hypophysectomized	
	No. of dead Inoculated	Mean day of death	Proportion with adrenal necrosis	Day of death	Proportion with adrenal necrosis
No drug	10/10	9.8	7/10	9, 10, 10	0/3
30 mg. per cent	9/10	13.0	8/9	17, . .	0/1
60 mg. per cent	3/10	21.7	2/3	18, . .	1/1*
90 mg. per cent	1/10	(18)‡	1/1	. . .	
120 mg. per cent	1/10	(13)	?	. . .	
	24/50		18/23		1/5*

* One animal incompletely hypophysectomized (testes and seminal vesicles only partially involuted) with adrenal necrosis.

‡ Figure in parenthesis value from single dead animal.

? One animal eaten by cage mates.

organs. They usually develop ophthalmitis (9) and sometimes encephalitis as a result of proliferating *Besnoitia*. However, after the 4 to 5th week of infection survivors generally become asymptomatic, save for residuals of earlier lesions. During the course of the subsequent months, hamsters die sporadically, usually following some weight loss. About one-half of these animals, show extensive adrenal necrosis as the only lesions of significance (Figs. 3, 8). Histologically, one finds extensive parasitization, only of adrenal cells, by proliferative *Besnoitia* with resulting necrosis and regeneration (Figs. 14 to 16). The other half of the animals die with a variety of lesions, of which renal para-amyloidosis and glomerular hyalinosis appear to be the most important.

Besnoitia cysts make their appearance during the 3rd week of infection and become visible in the gross during the 4th week. When intact they appear to exert little or no

chemotaxis. After cyst rupture an intense necrotizing inflammatory response follows, which apparently destroys all of the liberated organisms, since no evidence of parasitization of new cells can usually be found. To avoid cyst rupture as a complicating factor in pathogenesis, strains that have lost their cyst-forming capacity have usually been employed in this study, resulting in a more evenly progressing infection.

EXPERIMENTAL

Materials and General Procedures.—The majority of golden hamsters (*Mesocricetus auratus*) was purchased from the Lakeview Hamster Colony, Newfield, New Jersey. Hypophysectomies were performed through the very generous cooperation of Mr. Donald Otway of the Hormone Assay Laboratory, Chicago. It was found most expedient to use only young mature males so that testicular and scrotal atrophy could serve as an indicator of lost hypophyseal function, prior to their use.

Adrenocorticotrophin (porcine ACTH) was used in the purified form (National 395) containing 40 or 80 U.S.P. units per ml. in about 20 per cent gelatin. A large amount of aqueous material was supplied through the generosity of Dr. Gustav J. Martin, National Drug Company, Philadelphia. Compound A (11-dehydrocorticosterone acetate, 53R1721) and compound B (corticosterone acetate, 54R1177) were obtained through the courtesy of Drs. Harry J. Robinson and Karl Pfister of the Merck Institute, Rahway. Compound E (17-hydroxy-11-dehydrocorticosterone acetate, cortisone) and compound F (17-hydroxycorticosterone acetate, hydrocortisone, cortisol) were used as prepared commercially by Merck and Company. Compound S (11-desoxy-17-hydroxycorticosterone, 8419) and testosterone propionate U.S.P. (No. 8056) were obtained from the National Biochemicals Corporation, Cleveland. Each of the steroids supplied in bulk was homogenized in a glass tissue grinder and suspended in concentrations of 25 mg. per ml. in aqueous vehicle No. 1 (Merck 53R4527), a saline solution containing suspending agents and 0.9 per cent benzyl alcohol. In order to permanently mark the sites of steroid injections, a small amount of Higgins' India ink was added to the suspensions. DOCA (11-desoxycorticosterone acetate U.S.P.) was implanted in the form of pellets, obtained through the courtesy of Dr. Edward Henderson of the Schering Corporation and of Dr. Floyd S. Skelton formerly of this department. Epinephrine (adrenaline of Parke, Davis & Company, Detroit) was utilized in concentrations of 2 mg. per ml. of peanut oil. Drugs were administered subcutaneously into a clipped area on the rump of the animals.

Strains of *Besnoitia jellisoni* were maintained by intraperitoneal passage in mice. 5 or 10 per cent dilutions in saline of the mouse peritoneal exudate that developed were subinoculated twice weekly; the mice survived for 4 to 6 days. For experimental uses, hamsters were inoculated subcutaneously between the scapulae with approximately 0.2 cc. of a 10^{-2} suspension of mouse peritoneal fluid in normal saline (Abbott Laboratories No. 4210) unless noted otherwise. High passage strains, carried for many generations, and which no longer formed cysts, were generally used in order to study the direct effects of proliferative organisms, undisturbed by cyst rupture (9, 11). In most experiments, in which survival for prolonged periods was desired, sulfadiazine sodium in concentrations of 60 to 120 mg. per cent was administered in the drinking water for various periods of time, according to the length of protective effect desired. The water containing sulfadiazine was substituted for tap water 6 hours after inoculation of the organisms.

Animals were generally observed until moribund or dead. They were then autopsied, gross findings were noted, and tissues were fixed in Zenker-formol solution. Staining with periodic acid-Schiff and hematoxylin was used as routine, supplemented by other technics as needed. A complete report of microscopic studies will be made elsewhere. The Chi square test was applied to evaluate the numerical differences observed.

RESULTS

To study the effects of endocrine drugs on the development of adrenal necrosis during various stages of immunogenesis, two methods were used to provide infections that presented a less fulminant and more protracted course. First, the size of the inoculum was varied (Table I), and second, graded amounts of sulfadiazine were administered for various periods of time (Table II). Both procedures were applied in a parallel manner to groups of animals in which endocrine effects were to be compared. (Tables III to V).

Incidence of Adrenal Necrosis as Affected by Inoculum and Survival Period.—Table I shows the results of a titration in hamsters of peritoneal exudate from the 181st mouse passage. Adrenal necrosis, recognizable in the gross, was present in 77 per cent of animals that died. The minimal infective dose, which corresponded to a dilution of 10^{-5} , equalled the minimal lethal dose in these untreated animals; none of the titration survivors were found to resist homologous challenge 63 days later. In previous experiments, animals with chronic infections were found to be immune to challenge (11).

Adrenal Necrosis as Affected by Chemotherapy. Emergence of Immunity.—Table II demonstrates the suppressive effects on infection of a number of graded sulfadiazine dosages in unoperated and hypophysectomized hamsters. 60 mg. per cent of drug in the drinking water, given for 16 days, protected the majority of hamsters from death. The 50 per cent effective drug dose for unoperated female hamsters was 54 mg. per cent as calculated according to the Reed and Muench formula (20) and as plotted on probit paper. The incidence of adrenal necrosis amongst the fatalities was unaffected by sulfadiazine treatment; again 78 per cent of unoperated animals that died showed adrenal necrosis. It should be pointed out that unlike the survivors from titration (Table I), most of the survivors from treatment (Table II) appeared to remain infected. Between 60 and 200 days post infection, 8 of the 26 survivors died, 5 with adrenal necrosis; 17 of the 18 surviving beyond 200 days had developed cataracts, secondary to continuing ocular involvement with *Besnoitia* (9).

Completely hypophysectomized hamsters that died during the observation period of acute effects did not show adrenal necrosis. They appeared to survive infection while on prophylactic treatment as well as or better than, unoperated animals; this is further borne out in subsequent experiments. 6 of the 10 survivors died between 60 and 200 days after infection; none showed adrenal necrosis.

Effects of Hypophysectomy and of ACTH and Cortisone Administration on Adrenal Necrosis.—Table III illustrates the sparing effects on the adrenal gland of both hypophysectomy and ACTH administration.

The dosage of ACTH in groups P-159 and P-I4c was either 1 unit every other day, 1 unit daily, or 4 units daily for each of 2 hamsters: one such group of 6 hamsters was started on

ACTH treatment 5 days before infection, a second at the time of infection, and a third 5 days after infection. In group P-142, the ACTH dosage was constant at 5 units daily and the cortisone dosage at 2.5 mg. weekly; but the duration of sulfadiazine therapy was varied, 1 or 2 animals receiving chemotherapy for intervals ranging from 5 to 40 days. Of the 3 experimental

TABLE III
Effects of Hypophysectomy (Hypex), ACTH, and Cortisone Administration on B. jellisoni Infection. Acute Effects' Observation Period 62 Days

Experiment Group	Endocrine state	Total No.	Mortality			Surviving	Sulfadiazine		
			No. dead	Mean day of death	With adrenal necrosis		Treatment	Duration	
P-142	Unoperated	13	4	12	4	9/13	SD-90 mg. per cent	0, 5, 10, 15, 20, 30, 40 days	
	" ACTH	13	6	19	2	7/13	SD-90 mg. per cent	0, 5, 10, 15, 20, 30, 40, days	
	" cortisone	13	13	33	1	0/13	SD-90 mg. per cent	0, 5, 10, 15, 20, 30, 40 days	
	Hypophysectomy	6	1	13	0	5/6	SD-90 mg. per cent	15 days	
	Hypex + ACTH	10	1	22	0	9/10	SD-90 mg. per cent	15 "	
P-159	Unoperated	8	8	30	3/7	0/8	SD-90 mg. per cent	12 days	
	Hypex	6	2	30	0	4/6	SD-90 mg. per cent	12 "	
	" + ACTH	17	7	21	1	10/18	SD-90 mg. per cent	12 "	
P-14c	Unoperated	8	8	39	5	0/8	SD-120 mg. per cent	14 days	
	Hypex	8	6	40	0	2/8	SD-120 mg. per cent	14 "	
	" + ACTH	17	17	38	1	0/18	SD-120 mg. per cent	14 "	
Summary						No.	Mortality	With adrenal necrosis	Surviving
							per cent	per cent	per cent
Three groups combined	Unoperated.....					29	69	63	31
	Hypex.....					20	45	0	55
	Hypex + ACTH.....					44	57	8	41

groups, P-14c was a cyst-producing strain, whereas strains P-142 and P-159 consisted of proliferative forms only. Although the planned variables between the 3 strain groups affected survival rate and time, it is feasible to compare treatment effects by combining data from identically treated groups, especially those data relating to rates of mortality and the frequency of adrenal necrosis among the fatalities.

Unoperated animals showed the highest and hypophysectomized animals the lowest mortality ($p = 0.3$). Striking was the difference in the incidence of

adrenal necrosis; it reached 63 per cent amongst the unoperated hamsters (12/19), it was not observed in the hypophysectomized ones (0/9), and it was only 8 per cent in the ACTH-treated, hypophysectomized hamsters (2/25). The differences between the unoperated group and the 2 hypophysectomized groups are significant below the 1 per cent level, whether computed for total number inoculated or the fatalities only. The same rank relationships between treatment groups seen in the summary, are present within each strain group. ACTH-gel, 40 u/cc., 1 unit daily, maintained approximately normal adrenal size in the hypophysectomized hamsters, whereas 4 or 5 units per day frequently produced marked enlargement. There was no correlation between ACTH dosage and mortality; the two instances of adrenal necrosis in hypophysectomized animals occurred with a dose of 4 units per day. Whereas hypophysectomy and ACTH administration *decreased* rates both of mortality and of adrenal necrosis when compared to unoperated untreated animals, it is noteworthy that cortisone administration (P-142) *increased* the mortality rate (100 per cent) and *decreased* the incidence of adrenal necrosis (8 per cent). This phenomenon is analyzed in the next table.

Table IV depicts the experimental design that was used to evaluate the effects of cortisone and of other steroids on the course of infection. Large groups of hamsters were injected into the loose skinfold of the neck with a given dose of *Besnoitia*, they were then placed on a suppressive dose of sulfadiazine in drinking water. After 1, 2, 3, and 4 weeks, prophylactic sulfadiazine treatment was discontinued in subgroups of hamsters, and instead the drug to be tested was injected subcutaneously over the rump at regular intervals. In this manner the effects of corticoid and other drugs were tested during the entire course of early infection — before, during, as well as after the development of immunity.

Effect of Corticoids on Immunity and on Adrenal Necrosis.—Following the administration of cortisone acetate, 2.5 mg. twice weekly, the development of adrenal necrosis was suppressed almost completely (Table IV). After 2 to 3 weeks of prophylactic sulfadiazine treatment, normal hamsters were relatively immune, and deaths beyond the 26th day of infection were rare (Table II). However, cortisone-treated hamsters continued to die, even after 4 weeks of treatment, and the mortality rate reached 100 per cent, (Table IV). These results can be compared with those in Table II, in which animals received an identical inoculum, although drug dosage was varied, instead of duration of treatment.

When animals died after treatment with cortisone acetate, three findings were almost constantly observed: absence of necrosis of the adrenal gland (Fig. 11), necrosis and inflammation adjacent to the sites of subcutaneous cortisone acetate administration (Figs. 17 to 20), and confluent pneumonia with focal necrosis (Figs. 21 to 23). Sections through the areas of skin necrosis revealed marked focal proliferation of *Besnoitia* organisms, which was accompanied by cell destruction and inflammation (Figs. 18, 20). Giemsa-stained smears of the

TABLE IV
Effects of Cortisone (E Ac) and of 11-dehydrocorticosterone (A Ac) on Besnoitia Infection (P-181) of Hamsters
 10⁻⁷ given subcutaneously. Pretreatment with sulfadiazine (SD-60) for 1, 2, 3, or 4 weeks. Observation period 100 days. Each column refers to a single animal.

Unoperated															
Day	0-15		15-death		0-21		21-death		0-28						
	SD-60	E Ac	E Ac	E Ac	SD-60	E Ac	SD-60	E Ac	SD-60	E Ac					
Survival time, days	13	15	23	23	26	28	29	33	34	35	36	39	39	46	47
Adrenal necrosis	+	+	-	-	+	+	-	-	+	+	+	-	-	-	-
E Ac site inflammation	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Pneumonia, with Necrosis	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N

Hypophysectomized												
Day	0-15		15-death		0-21		21-death		0-28			
	SD-60	E Ac	E Ac	E Ac	SD-60	E Ac	SD-60	E Ac	SD-60	E Ac		
Survival time, days	11	13	24	30	30	31	33	36	38	41	43	45
Adrenal necrosis	-	+	-	-	-	-	-	-	-	-	-	-
E Ac site inflammation	P	P	-	+	?	?	+	?	+	-	?	?
Pneumonia, with Necrosis	P	P	-	P	N	N	N	N	N	N	N	N
Testes and seminal vesicle (Involved; if Uninvolved, hypophysectomy was incomplete)	I	U	I	I	I	I	U	I	I	I	I	I

Unoperated															
Day	0-7		7-death		0-14		14-death		0-28						
	SD-60	A Ac	A Ac	A Ac	SD-60	A Ac	SD-60	A Ac	SD-60	A Ac					
Survival time, days	11	22	25	27	32	38	S	S	S	14	14	14	14	14	14
Adrenal necrosis	+	?	-	-	-	-	+	+	+	-	-	-	-	-	-
Corticoid site inflammation	-	+	+	+	?	?	P	N	P	N	N	N	N	N	N
Pneumonia, with Necrosis	P	N	-	N	-	-	P	N	P	N	N	N	N	N	N

+ present, - absent, ? questionable, S surviving animal.

lesions demonstrated numerous *Besnoitia* organisms (Fig. 19). Cortisone acetate dissolved in the dehydrating alcohols, prior to sectioning. The areas where *Besnoitia* proliferated could, however, be related by their close proximity to the sites of cortisone injection, which was identified by finding the clefts in the loose connective tissue where cortisone acetate crystals had been deposited, or by the deposits of carbon, when India ink had been mixed with the cortisone inoculum. Control injections of India ink in aqueous vehicle No. 1 did not give rise to the skin lesions observed, nor did several other steroids, nor epinephrine in oil, as will be reported below.

The confluent pneumonia appeared likewise to result from proliferation of *Besnoitia*, since organisms were numerous throughout the lung, and were associated in very large numbers with the focal areas of necrosis (Figs. 22, 23).

Treatment of hypophysectomized hamsters with cortisone acetate resulted in similar proliferates of *Besnoitia* around the subcutaneous injection site; however, the incidence of these lesions was lower and the amount of inflammatory exudate was less. Adrenal necrosis was absent.

Treatment with compound A acetate tended likewise to suppress adrenal necrosis, to allow multiplication of *Besnoitia* at the subcutaneous injection site of the corticoid, and to give rise to pneumonia with focal necrosis. The tendency of compound A to produce late mortality was much less than that of cortisone; in the groups of hamsters that were treated, starting 14, 21, or 28 days after infection, and continuing up to the 46th day, only 1 out of 12 animals died. The 46 day mortality rate was 47 per cent. However, a group of three animals, which had been treated with compound A acetate from the 21st to the 46th day of infection, without mortality, died 12 to 16 days later when compound E acetate was substituted.

In hypophysectomized hamsters (not shown) the effects of compound A acetate administration were analogous to those observed with compound E acetate, but less pronounced than in the intact animals. The mortality ratio was identical to that in the unoperated compound A acetate-treated animals, and substitution of compound E acetate on the 46th day resulted in death within 10 to 15 days. Again, adrenal necrosis was not observed.

Effects of Other Steroids and of Epinephrine on Adrenal Necrosis.—Table V summarizes the results of an experiment in which the effects of steroids and other compounds were compared. The experimental design was essentially as indicated graphically in Table IV.

All the hamsters were inoculated on 1 day, they were then subdivided into groups of about 6 animals, which were to receive hormonal treatment at the end of 1, 2, 3, or 4 weeks. Animals surviving at least 1 day beyond the second injection of hormone (4 or 5 days after the first injection) are listed under the appropriate endocrine group; animals that died prematurely are listed together. Since the amount of compound B acetate available was sufficient for only 48 days of treatment, the tabulation was terminated on the 55th day. (Deposits of compound

B acetate could usually still be identified after 7 to 10 days at the site of subcutaneous injection).

Adrenal Necrosis.—Three groups can be distinguished relative to the incidence of adrenal necrosis. Whereas the latter was high in the India ink-injected control group (91 per cent), in the animals receiving testosterone (89 per cent)

TABLE V

Summary of Effects of Various Drugs on Besnoitia Infection (P-218b) of Hamsters (10⁻³ subcutaneously). Treatment starting 7, 14, 21, and 28 days after infection, repeated twice weekly.* Observation period 55 days.

Treatment group	No. in group	Mortality					Surviving per cent
		No. dead	Proportion with adrenal necrosis	Mean day of death	Proportion with		
					<i>Besnoitia</i> at Rx site	Necrotizing pneumonia	
India ink control.....	30	22	20/22	14	0	3/22	27
Premature deaths (a).....	54	54	51/54	15	0	6/54	0
Compound F, 2.5 mg. (b) -7, 0.....	12	12	1/12	9	0	0	0
(b) + 14, 21, 28.....	14	14	3/14	25	5/14	6/14	0
Compound B, 2.5 mg. intact.....	14	11	2/11	26	0	2/11	21
hypex.....	11	7	?2/11	32	0	0	36
Compound S, 2.5 mg. intact.....	15	9	6/9	20	0	0	40
hypex.....	11	4	?1/4	25	0	0	64
DOCA, 25 mg. (c).....	19	16	9/16	21	0	9/16	16
Testosterone, 2.5 mg.....	16	9	8/9	18	0	2/9	44
Epinephrine, 0.3 mg.....	16	10	7/10	25	0	3/10	38

Hamsters were unoperated, unless noted.

The acetates of compounds F, B, and S were given.

Hypex = hypophysectomized; intact = unoperated group of hamsters.

? Small focus of questionable necrosis, requiring serial sectioning for definitive diagnosis.

* Except as indicated below:

- (a) Animals that died prematurely before any test drug was given, including a few receiving only 1 injection, are listed separately.
- (b) Six groups of 6 hamsters were treated with F Ac., starting on days-7, 0, 14, 21, 28 relative to infection.
- (c) Single DOCA pellets of 25 mg. were implanted on days-5, 7, 14, 21.

and in the hamsters dying prematurely (95 per cent), it was low in the hypophysectomized groups (18-25 per cent), in the intact animals receiving compound B (18 per cent), and in the animals receiving compound F (8-21 per cent). The incidence of adrenal necrosis was intermediate in the animals receiving compound S (66 per cent), DOCA (56 per cent), and epinephrine (70 per cent). Qualitatively, the foci of necrosis were extensive, and frequently

accompanied by hemorrhage in the "high" and "intermediate" incidence groups; however, foci of necrosis were so small as to be doubtful entities, in the gross, in the hypophysectomized groups treated with compounds B and F. Temporally, adrenal necrosis was observed during the entire observation period in the "high" and "intermediate" incidence groups, but only within 5 days after beginning to administer compounds B and F.

Although the incidence of adrenal necrosis was markedly decreased, the mortality rate was 100 per cent in the animals treated with compound F, just as had been observed after treatment with compound E (Table IV). Mortality rates, in the range of the India ink control group, were also found in the unoperated animals treated with compounds B and S, testosterone, and epinephrine. The hypophysectomized group treated with compound S showed the only significant increase in survival rate.

Necrosis and Inflammation at Site of Glucocorticoid Injection.—Parasitization by *Besnoitia* of the medication injection site, with fibrinous exudate and necrosis, was observed in animals treated with compound F acetate, just as it had been described earlier for the injection sites of compounds E and A acetate (Fig. 19).

Although a granulomatous inflammatory reaction accompanied the deposits of peanut oil containing epinephrine, no *Besnoitia* organisms were found on sections. In a few instances, bacteria without significant inflammatory response were observed at the sites of DOCA deposits and of compounds B and S injection.

It was recently found that what had appeared to be a relatively solid immunity in the animals treated with compounds B and S, and in the India ink controls, was broken down within 16 to 22 days when treatment with compound E Ac, 2.5 mg. twice weekly, was substituted 90 days after infection. Organisms and cellular necrosis were observed around the sites of cortisone acetate deposition in some, and pneumonia with necrosis and organisms in all of these animals. This response is comparable to that illustrated in Table IV, in which immunity acquired during the administration of compound A Ac, was "challenged" by the administration of compound E Ac, subsequent to which all animals succumbed, showing a dissemination of organisms to the sites of E Ac injection, with proliferation occurring there and in the lung.

DISCUSSION

The Role of Steroids in the Pathogenesis of Adrenal Necrosis.—To facilitate discussion of the pathogenesis of the necrosis in the adrenal gland and at the medication site, as affected by a variety of endocrine states, the incidence of the lesions is graphically compared in Chart 1. Although taken from several experiments these can be compared qualitatively, and for the most even quantitatively as detailed in the legend.

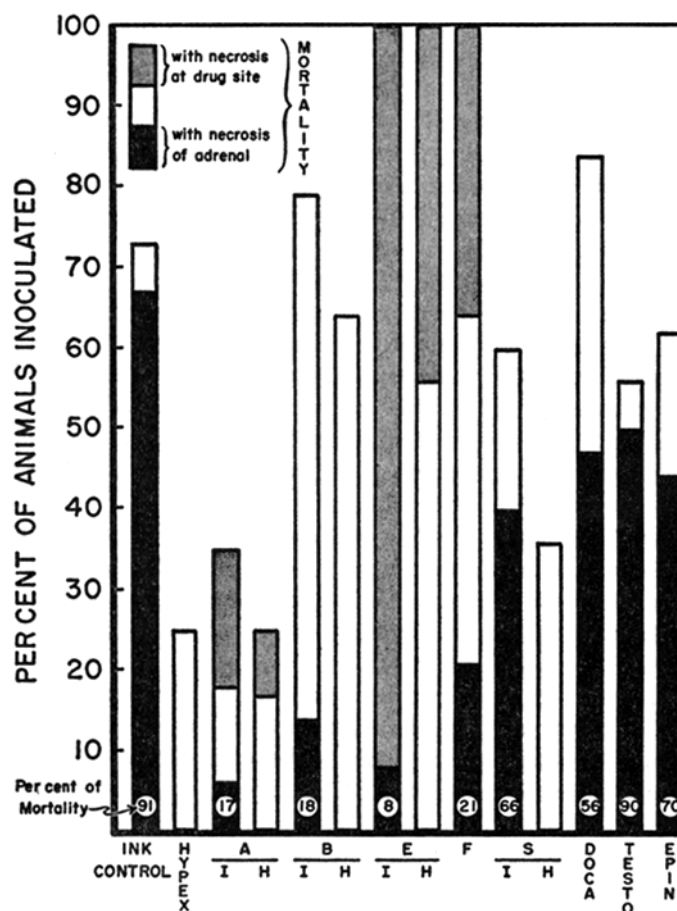


CHART 1. Mortality rates, incidence of adrenal necrosis and of necrosis at drug injection site. Abbreviations: A, B, E, F, S refer to the acetates of compounds A, B, E, F, S. I = Intact, H = HYPEX = Hypophysectomized hamsters. DOCA = desoxycorticosterone acetate, TESTO = testosterone propionate, EPIN = epinephrine in oil.

Each whole bar refers to mortality in terms of percentage of animals inoculated per treatment group; the incidence of adrenal necrosis is indicated by the length of the solid black portion, and the incidence of necrosis at the drug site, by the length of the stippled portion of the whole bar. The figures in circles refer to the percentage of adrenal necrosis in terms of animals that died. As in the preceding charts only data from the first 6 to 9 weeks are listed, to avoid inconsistencies due to termination or change of treatment, and due to sporadic late deaths from complications. Examination of the raw data did not indicate a significant difference of results whether the listings were cut off after 6 or 9 weeks, hence, they are given as in the charts.

Data for India ink control, compounds B, F, and S, DOCA, testosterone and epinephrine in oil are taken from one experiment summarized in Table V. Instances of questionable necrosis are excluded. Data for compounds A and E are from another experiment, summarized in Table IV. Data for the hypophysectomized (HYPEX) group are pooled from P-142 and P-159 in Table III. All data are derived from infections with non-cyst-producing *Besnoitia* strains. Pretreatment of animals with sulfadiazine turned out to have been more effective in the hypophysectomized group and the compound A-treated groups, hence their mortality rates are probably lower when compared to the others; the qualitative differences are not likely to have been affected, as indicated by comparison with the other simultaneously treated groups.

Although these data are taken only from animals that died, giving perhaps a distorted picture of the true incidence of adrenal necrosis, it is believed that surviving animals did not develop significant lesions of adrenal necrosis during the period of acute infection. This is based on examination of animals that died or were killed in the 4 to 6 week period, and the absence of significant scarring in the adrenals of animals dying later without showing adrenal necrosis.

Adrenal necrosis was observed in 91 per cent of control hamsters that died, and in a similar or slightly smaller incidence in hamsters dying while treated with testosterone (89 per cent) and epinephrine in oil (70 per cent). Hypophysectomy appeared to inhibit adrenal necrosis completely or nearly so. Certain of the corticoids inhibited adrenal necrosis markedly; compound A to 17 per cent, compound B to 18 per cent, compound E to 8 per cent, and compound F to 21 per cent of animals that died.

In view of the fact that in the few instances in which adrenal necrosis was found, this was observed generally within 4 to 5 days (compounds A, B, and F) and only once 11 days after the administration of corticoids, it appears possible that the adrenal necrosis might have started in the pretreatment period. These observations are therefore compatible with the interpretation that during the periods of administration of compounds A, B, E, or F, inhibition of adrenal necrosis was complete, or nearly so.

Since the administration of compounds A, B, E, and F caused adrenocortical involution, presumably due to a significant decrease in the production of ACTH, it is apparent that the lowered incidence, or absence, of adrenal cellular necrosis was accompanied by a decreased production, or absence, of ACTH-dependent corticoids in these cells. The results of the administration of exogenous corticoids on the adrenal are analogous to those of hypophysectomy and can therefore be attributed to a "chemical hypophysectomy" (Figs. 11 and 12).

The possibility that adrenal necrosis depends on the presence of certain corticoids within the adrenal cortex is made very likely by the observation that proliferation of *Besnoitia* leading to cellular necrosis occurred adjacent to the injection sites of some of the same corticoids that inhibited adrenal necrosis (Figs. 17 to 20).

The growth of *Besnoitia* may be assumed to depend largely on the conditions prevalent in their intracellular environment. Sufficient immunity to permit survival of the animal was acquired during the 3rd week of infection, as indicated by the survival of hamsters beyond the end of suppressive sulfadiazine treatment, which is illustrated in Tables II and IV. However, the injection of either compounds E or F into such immune animals again permitted the proliferation of *Besnoitia* adjacent to the injection sites, even 46 days (Table IV) or 90 days after infection (subsequent to the experiments described on Table V). Apparently, the corticoid acetate so modified local conditions that intracellular immunity was no longer effective.

It is interesting in this connection that the relatively soluble and diffusible compound A-alcohol did not bring about proliferation of *Besnoitia*, whereas the more insoluble compound A acetate, which persisted for 7 to 10 days at the subcutaneous injection sites, permitted proliferation at least during an early period of acquired immunity (1st week of infection, Table IV). Compared to compound A acetate, compound E acetate exerted a more potent immunity-depressing effect, observable even after immunity was seemingly well established.

Although little is known of adrenocortical secretion in hamsters all observations are consistent with the hypothesis that the secretory products include an immunity-depressing steroid, which permits more complete destruction of the adrenal cortex than of any other visceral organ of infected hamsters. This steroid resembles compounds E and F in their ability to permit the proliferation of *Besnoitia* after the appearance of immunity, rather than compounds A and B which allow proliferation only briefly or not at all.

Role of Steroids in Producing Pneumonia with Necrosis.—Pneumonia, predominantly exudative in character, is present in “unoperated” animals that die within a month after infection. Occasionally foci of necrosis are found in the lungs of such animals not treated with steroids (13 per cent). The incidence of focal to confluent areas of necrosis in the lungs due to proliferating *Besnoitia*, is increased progressively in groups treated with compounds A (33 per cent), F (43 per cent), E-hypophysectomized (78 per cent), and E-intact (100 per cent) (Figs. 21 to 23). Since focal pulmonary necrosis was not observed in untreated hypophysectomized and compound A-treated hypophysectomized animals, it appears likely that excessive proliferation of *Besnoitia* within the lungs, leading to confluent cellular necrosis, is likewise dependent on the effect of immunity depression by corticoids, compound E being the most effective.

Tissue Immunity.—The gradual appearance of immunity during the 2nd and 3rd weeks of infection has been mentioned. As discussed also, it is during this period that continued proliferation of *Besnoitia* can be observed in the adrenal cortex. The adrenal glands are between normal and twice normal size and necrosis is focal to confluent. During the subsequent weeks and months, proliferation of *Besnoitia* can be reactivated around subcutaneous deposits of injected compounds E and F, in the regional lymph nodes, as well as in the lungs of such animals, causing necrosis of parasitized cells at either site.

About 5 or 6 months after infection, hamsters are found to die sporadically, with adrenals enlarged five, ten or more times normal size and with evidence of chronic progressive necrosis, inflammation, and regeneration (Figs. 3, 8). Inflammatory exudate usually surrounds the adrenal cortex, but it stops abruptly at the surface of the attached kidneys, liver, or spleen, if they have become adherent (Figs. 14 to 16). Lesions due to the active proliferation of

Besnoitia are found only in the adrenal glands. This fact again points to the exceptional susceptibility of the adrenals, in an animal whose other organs are relatively resistant as a result of acquired immunity.

The finding that the adrenal medulla is generally involved to a degree similar to that found in the cortex, may be due to the fact that medullary cells are bathed in steroid-containing blood draining into the central adrenal vein. It has previously been discussed that the increased content of corticoids in the lung and lymph nodes (the first organs filtering the venous and lymphatic drainage from the injection site) may also account for the proliferation of organisms there, rather than in the liver and spleen, which were as highly susceptible in the non-immune animal but are supplied by blood that has been filtered through the lung.

A patient with Addison's disease was observed recently (27) from whose blood stream *Histoplasma* was cultured repeatedly. In spite of this parasitemia, progressive lesions due to *Histoplasma* were found only in a few areas at autopsy. The adrenals were largely necrotic owing to parasitization of cells and to infarction (Fig. 4). They were greatly enlarged owing to the inflammatory exudate and the regeneration of cortical cells. The liver close to the adherent right adrenal was parasitized by *Histoplasma*, but the remaining liver was free of lesions. Since the central adrenal vein was thrombosed, the venous drainage from the remaining viable islands of cortical cells went peripherally to the liver. The broad band of connective tissue between the adrenal and the adherent kidney was parasitized, but the kidney was not. In spite of hematogenous dissemination, local factors appeared to determine where *Histoplasma* was able to proliferate. One of these factors appeared to be whether or not cells elaborated, or were bathed in blood containing adrenal corticoids. Other progressive lesions were present in the mucosa of the ileum, and in the brain. The lungs, spleen, lymph nodes, and bone marrow, which are extensively involved in disseminated histoplasmosis, contained only insignificant active lesions in our patient. Analogous situations have been found in other cases of Addison's disease due to histoplasmosis and tuberculosis (11).

It has been mentioned that in the hamster infected with *Besnoitia*, immunity is incomplete in the sense that infection persists in animals surviving the acute infection. Since organisms can be isolated in small numbers from a variety of organs, it is possible that they persist also in the adrenals and proliferate slowly causing cellular necrosis, which eventually reaches a rate exceeding that of cell regeneration. By migrating from cell to cell, organisms need not come in contact with the blood stream which contains antibody. That parasitemia can occur, at least after the administration of exogenous cortisone, is indicated by finding organisms in the blood stream, and around the subcutaneous injection sites, as late as 106 to 112 days after infection, and 16 to 22 days after the onset of treatment with cortisone acetate.

Effects of Hypophysectomy.—Adrenal necrosis was rarely encountered in hypophysectomized hamsters. This would be expected if corticoids are necessary to support proliferation of *Besnoitia* in an immune host. Rare instances

of necrosis might be due to the hormonal effect of a small remnant of pituitary gland, the presence of which might be difficult to prove or exclude, unless gonadotropins are also secreted. Hypophysectomized animals developed smaller or no lesions at the subcutaneous injection site, and they survived infection better than intact animals. However, their water intake, and hence their sulfadiazine dosage was higher, which might explain their lesser morbidity and mortality. Nonetheless, even after termination of sulfonamide therapy, fewer animals died, and lesions at the corticoid injection site were fewer and smaller. It is possible, that the lack of other pituitary hormones, especially growth hormone, modified the course of infection. This is being investigated.

Effects of Exogenous ACTH on the Hamster Adrenal.—As shown in Table III, hypophysectomy prevented adrenal necrosis, and the administration of exogenous ACTH permitted it to occur in only 8 per cent of animals that died. In unoperated animals (P-142), ACTH administration decreased the incidence of adrenal necrosis from 4 out of 4, to 2 out of 6 animals that died. Compared to an over-all incidence of adrenal necrosis of 63 per cent in unoperated hamsters, the decrease was marked ($p = 0.01$) in the hypophysectomized ACTH-treated animals, but only suggestive ($p = 0.13$) in the unoperated ACTH-treated hamsters. Whereas both the "stress" of infection and ACTH administration increased adrenal size, these findings seem to suggest a difference in the mode of action of endogenous and exogenous ACTH on the adrenal gland.

It has been suggested that flushing out adrenal corticoids due to excessive stimulation by exogenous ACTH might prevent necrosis from occurring. This appears unlikely, since adrenal necrosis occurred in hypophysectomized hamsters only when treated with 4 units but not with 1 unit of purified ACTH-gel daily.

It has also been suggested that microorganisms might invade and proliferate in functionally active cells in preference to dormant ones, and that a change in susceptibility and vulnerability of adrenal cells might not necessarily be due to a high concentration of steroid, but rather might be due to the high metabolic activity. Since no direct measurements of metabolic activity are available this question must remain essentially unanswered. However, if the enlargement of adrenal cortical cells and the increase in thickness of the cortex (Figs. 10 and 13), which follows the administration of exogenous ACTH, can be taken as an indication of metabolic activity, then the proliferation of organisms is either little or adversely affected by it. At the sites of subcutaneous deposits of compounds E, F, and A, where proliferation of *Besnoitia* is facilitated, it is difficult to postulate increased metabolic activity, other than that mediated by these corticoids. It is possible that a "compound P," which facilitates proliferation, exists in the adrenals of untreated and "stressed" hamsters, which

is reduced in amount by exogenous ACTH and markedly inhibited by therapy with compounds A, B, E, and F. At this time it is only possible to state that if such a "compound P" exists, it resembles compounds E and F in permitting proliferation of *Besnoitia*.

Kass, Hechter, Macchi, and Mou (16) showed that the corticoid content in the adrenal vein blood of rabbits shifted slowly from predominantly compound B to F under the influence of prolonged administration of porcine ACTH. It appears possible that also in the hamster the adrenal secretory product changes under the influence of exogenous ACTH. Whether this is due to a species difference in ACTH, is not yet evident. Porcine ACTH, while increasing the size of the hamster adrenal cortex, may affect the presence of the 17-hydroxylating enzymes (4), thereby changing the nature of the secretory product. If one can apply the data from the hypophysectomized animals (although the roles of growth and other hormones have not yet been investigated), explanations based on the quantity of ACTH or its possible flushing-out effect would appear to be excluded, and species differences in the chemical activity of hamster *versus* hog ACTH might best explain the fact that exogenous ACTH depresses the incidence of adrenal necrosis. Such species differences have been suspected also on the basis of other evidence by DiRaimondo, Orr, Island, and Forsham (3).

In view of the susceptibility of the hamster adrenal to prolonged parasitization by *Besnoitia* during the emergence of immunity, it is likely that one of the native adrenocortical secretory products is resistance-depressing, similar to compounds E and F. Since under the influence of porcine ACTH the hamster adrenal appears to become more resistant, it is possible that a shift towards the production of a less resistance-depressing compound, such as A or B takes place. This matter is being further investigated.

The observations by Shwartzman and Fisher (22, 23) that cortisone, but not ACTH, decreases resistance of hamsters to type II poliomyelitis virus, is consistent with the hypothesis presented here. Although it was not observed that ACTH-treated animals were more resistant, this may have been due to the more infrequent administration of ACTH. However it appears significant that morbidity was decreased slightly after unilateral and significantly after bilateral adrenalectomy (24). Other examples of a discrepancy in activity of ACTH and cortisone on infection have been listed by Kass and Finland (15).

Effects of Other Steroids.—The incidence of adrenal necrosis (expressed as per cent of mortality) was similar in the control group, and the groups treated with testosterone, adrenalin, and compound S. Only the DOCA-treated group showed what might be a significant decrease ($p = 0.04$). The six hamsters that had been pretreated, starting 5 days prior to infection, showed the longest survival, presumably in part due to their greater water and, hence, sulfadia-

zine intake. Although only 3 of their 12 adrenals showed necrosis, proof of an inhibitive effect of DOCA on the development of adrenal necrosis awaits confirmation under conditions in which the sulfadiazine treatment is better controlled, either by feeding or by injection.

Comparison of Animal Model and Adrenal Necrosis in Man.—As was mentioned in the introduction, adrenal necrosis occurring during chronic *Besnoitia* (and *Toxoplasma*) infection of hamsters was considered comparable to chronic tuberculosis and histoplasmosis of the adrenal gland in man. The present study deals with adrenal necrosis during the acute, subacute, and early chronic *Besnoitia* infection, since the earlier disease lends itself better to experimental manipulation than the more sporadic instances of morbidity and mortality later-on. It is presumed that the pathogenesis of the late adrenal lesions is similar to the early lesions, depending primarily on the modifying effect on cellular immunity, of a resistance-depressing corticoid, permitting localized proliferation of *Besnoitia* in a host with lesser or greater degrees of acquired immunity.

If this hypothesis is correct, certain postulates can be made on the basis of the animal experiments, which might have bearing on the human disease in those instances in which Addison's disease is secondary to microbial destruction of the adrenal gland.

(a) In order to protect the remaining adrenal cortex and to preserve it for possible regeneration after the infection has been overcome, it would appear desirable to inhibit secretion of the resistance-depressing adrenal corticoid, which in man appears to be largely compound F (2, 14).

(b) Steroid therapy for purposes of hormone replacement and secretory inhibition should preferably be conducted with compounds that have a minimal resistance-depressing activity.

On the basis of the data compared in Chart 1, both cortisone and hydrocortisone, now in general use, would appear undesirable, since although depressing the production of endogenous steroid, they also show marked resistance-depressing activity in pharmacologic doses. The same objection would apply to 11-dehydrocorticosterone (compound A), although to a lesser degree, and to prednisone and prednisolone which in recent experiments showed activities comparable to compounds E and F (11). In these experiments, the only compound with glucocorticoid and adrenal-inhibitory activity that did not depress resistance, was corticosterone (compound B). This compound, which appears to be one of the natural secretory products of the human adrenal (14), has been used with satisfactory results (25) in the treatment of Addison's disease prior to the availability of cortisone, but was replaced by the latter because of its greater glucocorticoid activity (1, 25). The data presented here might justify reconsideration of the relative glucocorticoid and resistance-depressing activities of the various steroids. Clinical experimentation should determine their relative usefulness, which may be different in man than in the hamster, and which would depend

in part on the availability of antimicrobial therapy, such as for tuberculosis, or the virtual lack thereof, such as for histoplasmosis.

Comparison of Microbial Necrosis of the Adrenal Gland with Toxic "Hemorrhagic Adrenal Necrosis".—The Waterhouse-Friderichsen syndrome accompanying meningococemia in man, and the intense adrenal congestion accompanying death from diphtheria intoxication in guinea pigs are well known examples of what has been termed "hemorrhagic adrenal necrosis." The histologic picture is more often that of congestion and hemorrhage rather than of necrosis, although necrosis has been observed secondary to what appears to be cytotoxicity, and secondary to thrombosis of the central adrenal vein. The investigations of Tonutti, summarized recently (26), have shown that the adrenal reaction to diphtheria toxin in guinea pigs depends on the presence of the pituitary gland or of exogenous ACTH. Diphtheria toxin appears to be innocuous to the adrenals of animals hypophysectomized as late as 10 hours after toxin administration. Nonetheless, these animals die from general intoxication, and even somewhat earlier than unoperated guinea pigs. Administration of cortisone, however, extends their survival period to that of normal animals. In unoperated animals the adrenals of which were passively protected by antitoxin given intraperitoneally, diphtheria toxin injected intracerebrally gave rise to signs of ACTH action on the adrenals, without, however, leading to hemorrhage and necrosis. Tonutti concludes that development of the lesions depends both on the non-specific stress effect and on the specific action by diphtheria toxin on the adrenal cortex. He also points out that certain toxins give rise only to stress reactions, whereas others, such as meningococcal toxin, give rise to hemorrhagic necrosis even in hypophysectomized guinea pigs.

The guinea pig diphtheria intoxication model superficially resembles the hamster-*Besnoitia* infection model in that ACTH appears necessary for adrenal lesions to occur in both instances. However important differences are apparent. *Besnoitia* infection is characterized by cellular necrosis due to microorganismal growth (Fig. 9), while vascular congestion and hemorrhage do not become prominent unless exogenous ACTH is administered (Fig. 10). In diphtheria intoxication, the adrenal reaction results after a fixed latent period irrespective of the quantity of toxin given, and when necrosis occurs, it appears to be secondary to the intense vascular congestion. Diphtheria toxin gives rise to lesions only in the non-immune guinea pig, whereas *Besnoitia* proliferation destroys corticoid-conditioned cells even in a partially immune host. There have been no reports, in spite of many observers (5, 18, 21), that cortisone injected into guinea pigs, sensitizes the site of injection to the action of diphtheria toxin; however cortisone does render cells more susceptible to *Besnoitia*. Exogenous

cortisone inhibits adrenal necrosis due to *Besnoitia*, and there is suggestive evidence that it might also inhibit adrenal congestion when adequate doses are given to guinea pigs and to toxin-treated mice (18). It might be postulated that ACTH produces a substance within the adrenal cortex, which renders the latter susceptible to diphtheria toxin and to microbial proliferation. However, it has been found (11) that the hamster adrenal can be infected even in a hypophysectomized animal, provided acquired immunity is absent and *Besnoitia* organisms reach the adrenals. The phenomenon described in this paper refers to the selective localization and proliferation of organisms in an otherwise immune host.

Immunologic and Endocrine Balance during Infection.—Dealing with a specific infection, the direct effects of the micro-organism are preponderant over the modifying, non-specific stress effects of infection. The conditioning factors of early infection, resulting in humoral and cellular immunity, tend to establish a balance with the immunity-depressing corticoid effects. When the latter effects predominate, proliferation of organisms is possible. This may occur in the corticoid-secreting adrenal, both early and late during the natural course of the infection, or, at various times adjacent to certain experimentally deposited corticoids. How these corticoids permit increased proliferative activity of *Besnoitia* to occur, has not yet been established. To attribute it to their “anti-inflammatory” action would be an over simplification, since a significant inflammatory response is present (Figs. 17 to 20). During recent years, the generalized immunity-lowering effects of exogenous corticoids have been observed frequently, both experimentally and clinically (15). An experimental model demonstrating the effects of regional hypercorticoidism on infection has now been added. The recognition thereof allows formulation of a hypothesis which could serve to explain the pathogenesis of microbial necrosis of the adrenal, that leads to Addison’s disease in man.

SUMMARY

Adrenal necrosis has been described in golden hamsters where it occurs during the course of infection with *Besnoitia jellisoni*. This necrosis results directly from the active intracellular proliferation by this obligate intracellular protozoan organism.

After infection, adrenal necrosis is rarely observed in hypophysectomized hamsters. In unoperated animals adrenal necrosis is suppressed to varying degrees by cortisone (E), hydrocortisone (F), corticosterone (B), 11-dehydrocorticosterone (A), and possibly by 11-desoxycorticosterone (DOCA).

Besnoitia organisms proliferate in otherwise “immune” hamsters around the subcutaneous deposits of the acetates of cortisone (E), hydrocortisone (F), and

11-dehydrocorticosterone (A); a marked depression of general immunity follows the administration of pharmacologic doses of the former two hormones. Organisms do not proliferate around the sites of corticosterone acetate (B) and 11-desoxycorticosterone acetate (DOCA) injection, nor next to deposits of testosterone propionate, 11-desoxy-17-hydroxy-corticosterone acetate (Reichstein's compound S) and epinephrine in oil.

It is postulated that certain glucocorticoids can so modify immunity mechanisms locally, that general immunity becomes ineffective; this occurs in the adrenal glands owing to endogenous corticoid production, at the sites of exogenous corticoid injection, and proximal to that in the lungs.

A comparison is made with the pathogenesis of tuberculosis and histoplasmosis of the adrenal gland which results in Addison's disease in man, and it is concluded that a similar pathogenetic mechanism is operative. The use of glucocorticoids for replacement therapy is discussed in reference to their relative resistance-depressing activities in pharmacologic doses. These undesirable side effects would appear to be less pronounced, if not absent, if corticosterone (B) rather than cortisone (E) and hydrocortisone (F) therapy were used.

Porcine adrenocorticotrophic hormone (ACTH) appears to depress the incidence of adrenal necrosis in unoperated hamsters, and supports proliferation of organisms in the adrenal cortex with subsequent necrosis in only a small proportion of hypophysectomized hamsters. The possibility is discussed that ACTH from a different species (hog) might lead to a change in the secretory activity of the hamster adrenal gland.

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EXPLANATION OF PLATES

PLATE 13

Gross appearance of adrenals and kidneys in normal and diseased states. Figs. 1 to 3 are from unfixed organs, Figs. 5 to 8 from fixed hamster organs, all magnified 1.5. Fig. 4 shows fixed organs from a patient with Addison's disease due to histoplasmosis. Note that in both animals and human being the necrosis is confined to the adrenal gland and does not extend into the adherent organs. Abbreviations: A = adrenal, K = kidney, L = liver, S = spleen, G = gallbladder.

FIG. 1. Normal *in situ* appearance of hamster adrenals, above, and kidneys, below.

FIG. 2. *Besnoitia* infection of 18 days' duration. *In situ* view. Multiple light areas indicate necrotic foci in slightly enlarged adrenal glands.

FIG. 3. *Besnoitia* infection of 251 days' duration. Very much enlarged right adrenal, apparently completely necrotic, to which the right lobe of the liver has become adherent by an inflammatory exudate.

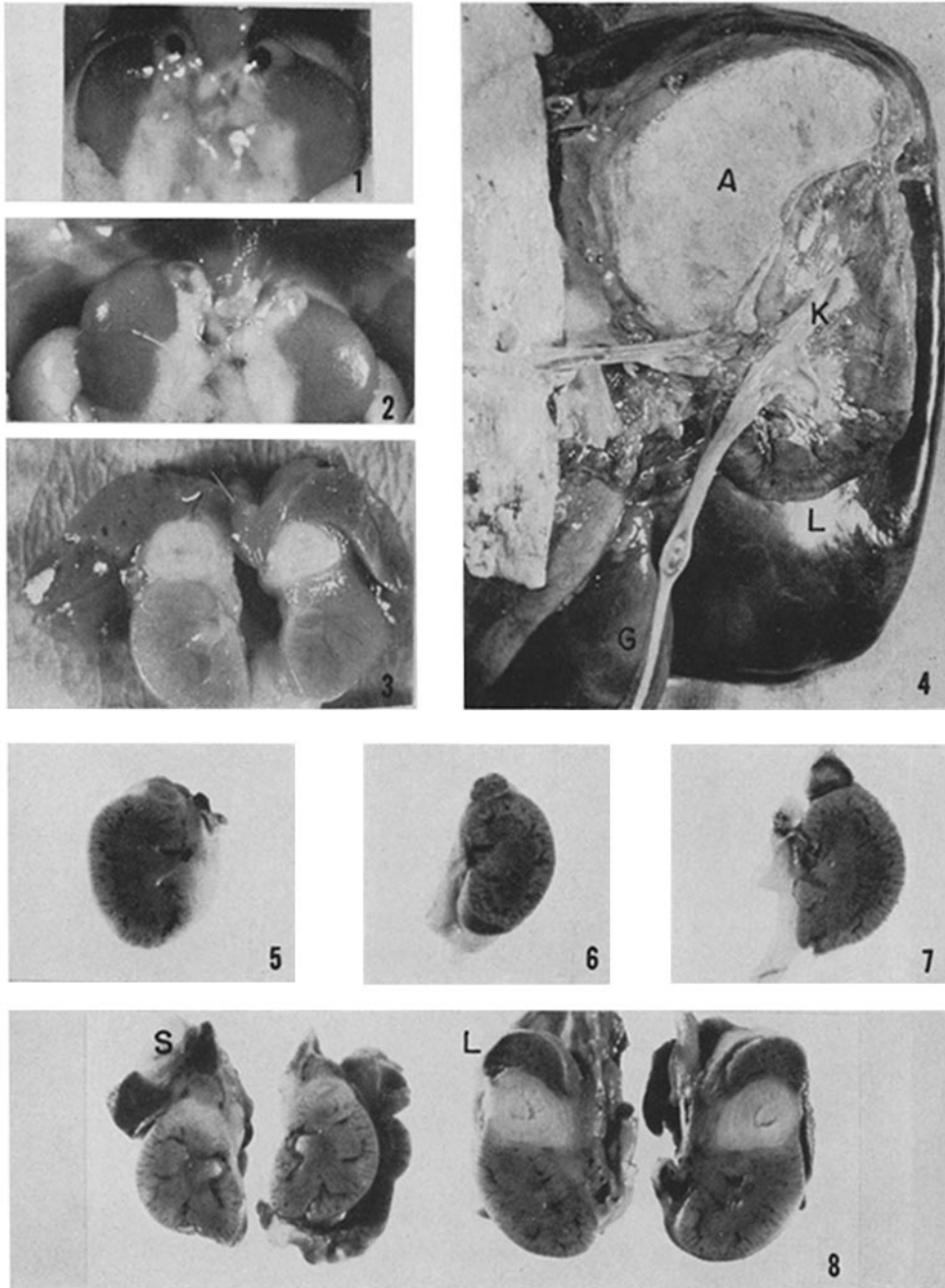
FIG. 4. Posterior aspect of greatly enlarged ($9 \times 5 \times 4$ cm.) and almost completely necrotic right adrenal gland from a patient with Addison's disease due to histoplasmosis (KUMC autopsy 715). The left adrenal was of similar appearance. Reduced to about one-half of actual size.

FIG. 5. Normal appearance of bisected kidney and adrenal gland (above) of hamster.

FIG. 6. *Besnoitia* infection of 11 days' duration. Adrenal gland with light areas, indicating necrosis, and dark areas indicating congestion and hemorrhage. Fig. 9 is from same adrenal gland.

FIG. 7. *Toxoplasma* infection of 1 year's duration. Enlarged adrenal gland with necrosis and hemorrhage.

FIG. 8. *Besnoitia* infection of 175 days' duration. Bisected right and left adrenal glands showing enlargement and necrosis. Liver and spleen have become adherent by inflammatory exudate. See Figs. 14 to 16 for their microscopic appearance.



(Frenkel: Hormones and adrenal necrosis)

PLATE 14

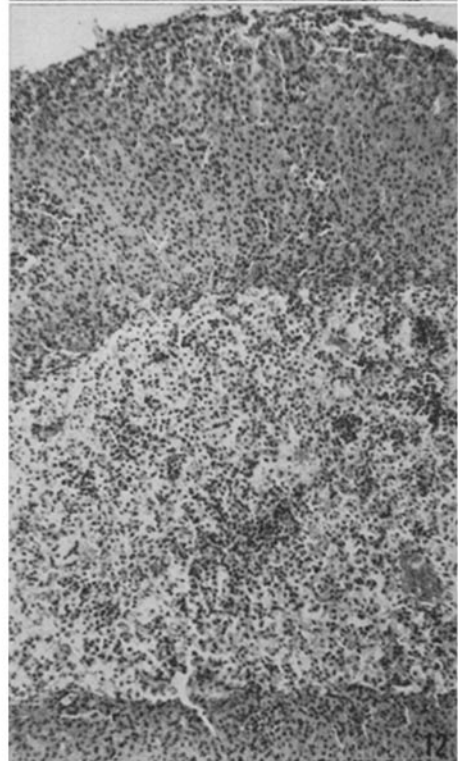
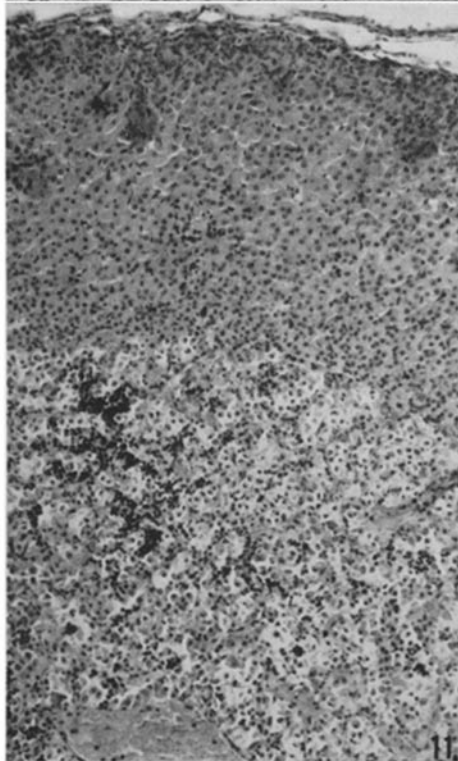
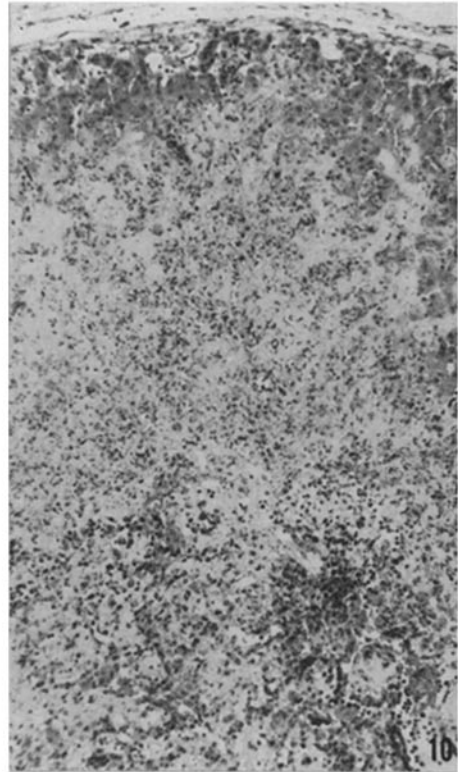
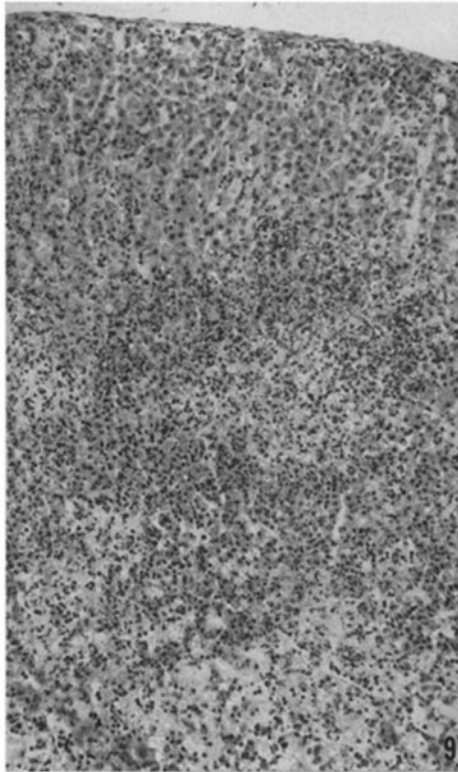
Relation of endocrine states to the microscopic appearance of the adrenal glands of hamsters, with acute *Besnoitia* infection. Sections pass through the centers of the adrenal glands and give an indication of the thickness of the cortex. Zenker-formol (ZF) fixation, stained with hematoxylin and eosin (H + E), magnification 100. Treatment, if any, is indicated for each animal.

FIG. 9. *Unoperated*, no exogenous hormone. Cellular necrosis and inflammation centered in the adrenal cortex due to proliferation of *Besnoitia*. Duration of infection 11 days, treated with 60 mg. per cent of sodium sulfadiazine in drinking water for 7 days. Same adrenal as illustrated on Fig. 6.

FIG. 10. *Unoperated*, *ACTH 5 units daily* $\times 14$. The appearance is similar to that in Fig. 9, however congestion and hemorrhage are more intense, and the intact cortical cells are larger. For close-up see Fig. 13. Duration of infection 15 days, treated with 90 mg. per cent of sodium sulfadiazine in drinking water for 5 days.

FIG. 11. *Unoperated*, *cortisone acetate 2.5 mg. weekly* $\times 3$. Note absence of necrosis, narrow cortex, small cell size, infiltration of lymphocytes into inner cortex and medulla. *Besnoitia* organisms not seen in the adrenal; the immediate cause of death was pneumonia due to *Besnoitia*. Figs. 22 and 23 show the lungs from the same animal. Duration of infection 15 days, treated with 90 mg. per cent of sodium sulfadiazine in drinking water for 5 days.

FIG. 12. *Hypophysectomized for approximately 6 weeks*, *no exogenous hormone*. Note absence of necrosis, narrow cortex, small nuclear and cell size, infiltration of lymphocytes into inner cortex and medulla. *Besnoitia* not seen in the adrenal; lesions were present at the injection site, the lungs, liver, and spleen. Duration of infection 13 days, treated with 90 mg. per cent sodium sulfadiazine in drinking water for 13 days.



(Frenkel: Hormones and adrenal necrosis)

PLATE 15

Selective localization of proliferative *Besnoitia* organisms and of resultant cellular necrosis in the adrenal gland (bottom), without involvement of the adjacent organ (top).

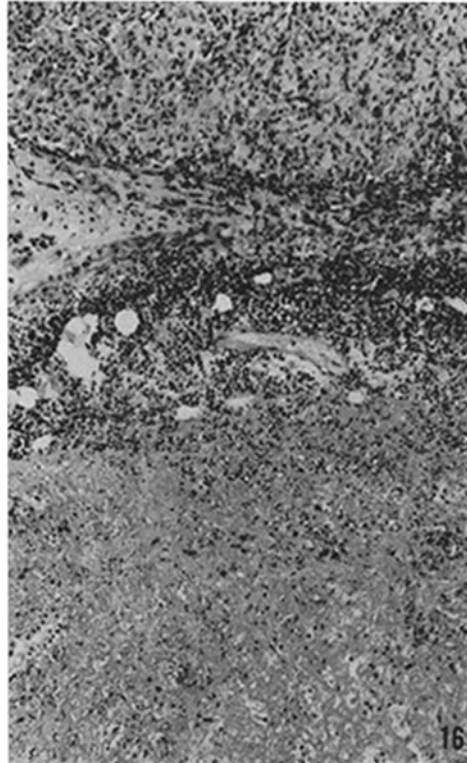
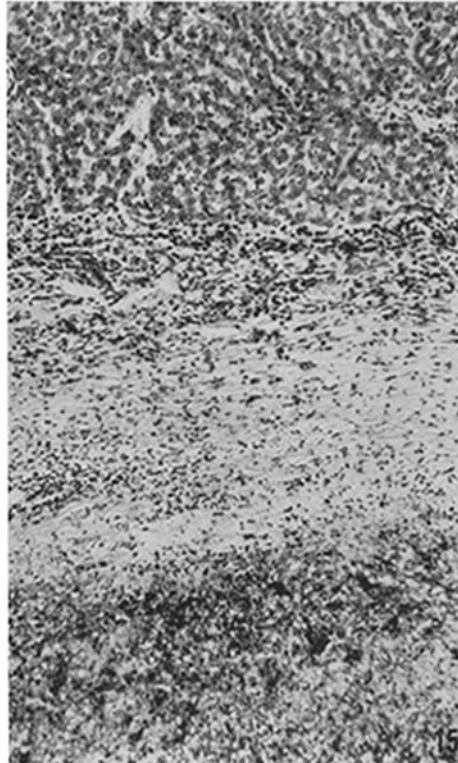
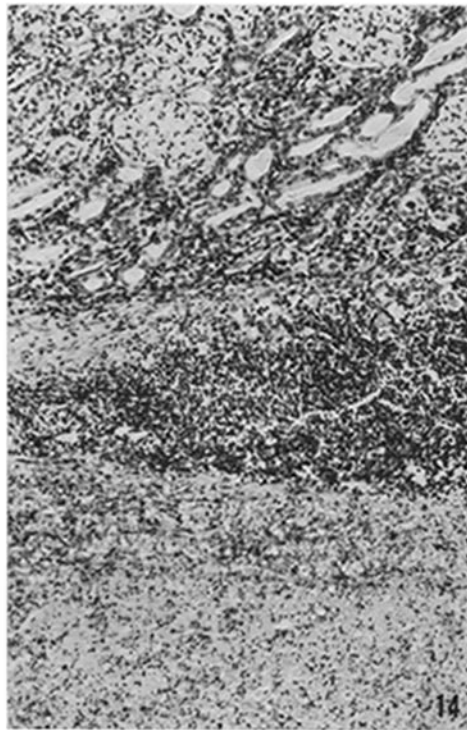
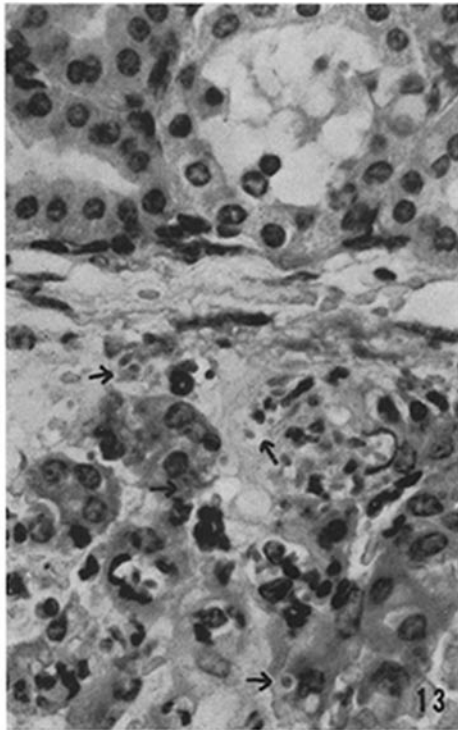
FIG. 13. *Acute infection, duration 15 days.* *Besnoitia* organisms are indicated by arrows in and between necrotic adrenal cortical cells. Kidney is uninvolved. Sulfadiazine treatment 90 mg. per cent in water for 5 days, treated with ACTH; same adrenal as shown in Fig. 10. ZF, H + E. $\times 560$.

Chronic infection, duration 175 days. Nearly complete necrosis of the adrenal gland. See Fig. 8 for gross appearance; sulfadiazine treatment 120 mg. per cent in water for 16 days. ZF, H + E. $\times 100$.

FIG. 14. Adrenal necrosis with kidney showing glomerular hyalinosis, tubular casts and chronic inflammation extending from adrenal. Note absence of necrosis in kidney.

FIG. 15. Adrenal necrosis with liver adherent by organized inflammatory exudate. Lobular architecture of liver is intact; there is slight mononuclear cell infiltration.

FIG. 16. Adrenal necrosis with spleen adherent by organized inflammatory exudate. The splenic parenchyma contains amyloid-like deposits and is depleted of lymphoid elements.



(Frenkel: Hormones and adrenal necrosis)

PLATE 16

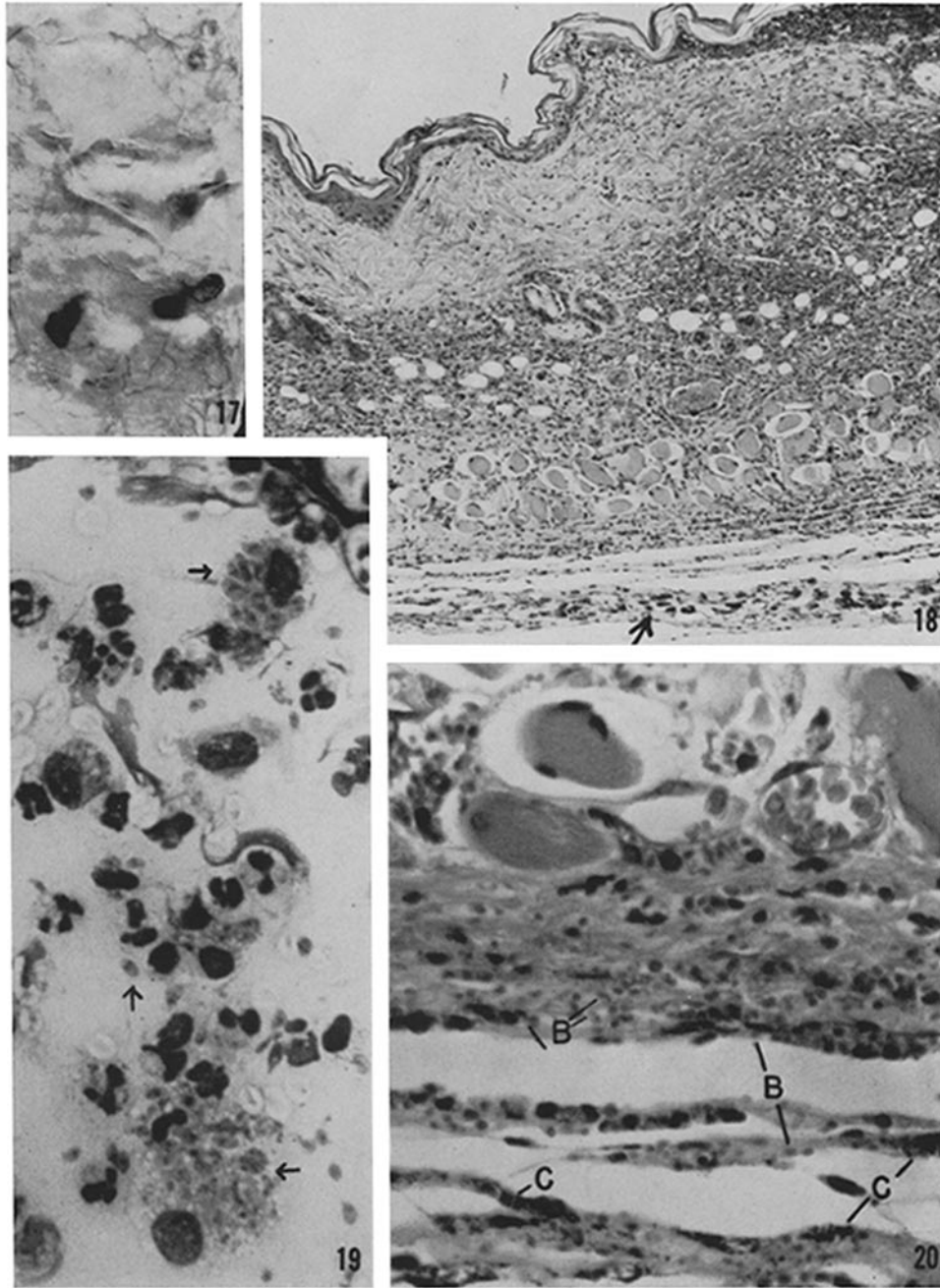
Proliferation of *Besnoitia*, with inflammation and necrosis at the site of the subcutaneous injection of corticoids. Figs. 17, 18, and 20 are from the same hamster injected with cortisone acetate; Fig. 19 from an animal injected with hydrocortisone acetate.

FIG. 17. Inner aspect of formalin-fixed skin. Four deposits of cortisone acetate are marked by India ink. They are surrounded by whitish fibrinous exudate. *Besnoitia* infection was followed by 28 days of sulfadiazine treatment (60 mg. per cent), and in turn by 5 injections of 2.5 mg. of compound E acetate, given at 3 day intervals between days 29 and 46, at which time the hamster died. $\times 1$.

FIG. 18. Section through skin showing the cortisone acetate deposits in the subcutaneous connective tissue marked by India ink (arrow). Much necrosis and inflammation due to the proliferation of *Besnoitia* organisms is present in the dermis, subcutaneous muscle layer and connective tissue, which led to ulceration, upper right. ZF, H + E. $\times 100$.

FIG. 19. Macrophages, neutrophil granulocytes, and intra- and extracellular *Besnoitia* (arrows) are numerous on smear stained with Giemsa's stain from site of injection of hydrocortisone acetate (2.5 mg. twice weekly starting 15th day); 32 days after infection; treatment with 60 mg. per cent sodium sulfadiazine for 24 days. $\times 560$.

FIG. 20. Section through skin showing numerous *Besnoitia* organisms (B) in the subcutaneous connective tissue and adjacent to cortisone acetate deposits marked by carbon pigment (C). Degenerating skeletal muscle in upper portion of picture. From near left lower corner of figure 18. ZF, H + E. $\times 560$.



(Frenkel: Hormones and adrenal necrosis)

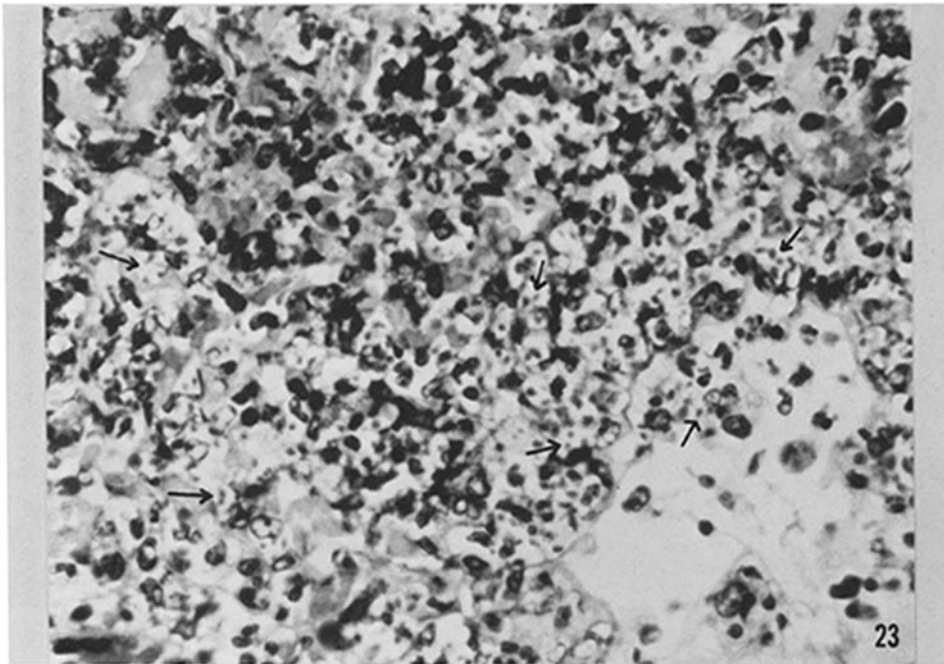
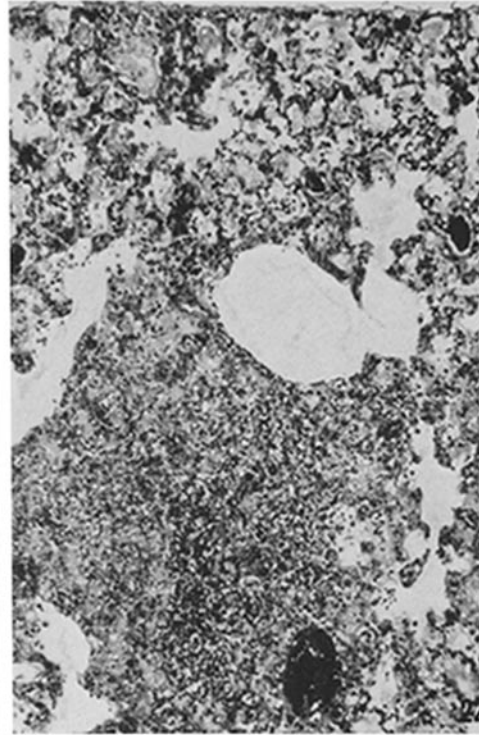
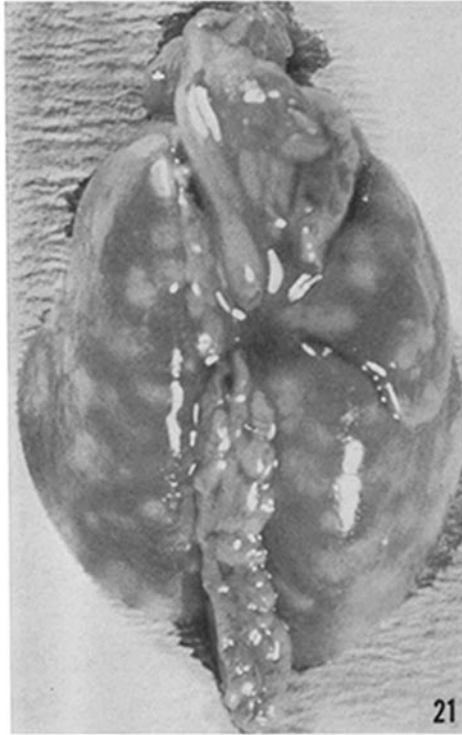
PLATE 17

Pneumonia with necrosis associated with the administration of pharmacologic doses of exogenous corticoids in hamsters infected with *Besnoitia*.

FIG. 21. Dorsal view of lungs with confluent pneumonia and whitish areas indicating inflammation with necrosis. Duration of infection 210 days; treated with cortisone acetate 2.5 mg. twice weekly from the 191st day to death.

FIG. 22. Diffuse pneumonia with focus of intense inflammation and necrosis. Duration of infection 15 days, treated with sulfadiazine for 5 days and with 2.5 mg. of cortisone acetate on the 1st, 7th, and 14th day of infection. See Fig. 11 for appearance of the adrenal gland of this animal. ZF, H + E. $\times 100$.

FIG. 23. *Besnoitia* organisms (arrows) are numerous in areas of necrosis shown on FIG. 22. $\times 560$.



(Frenkel: Hormones and adrenal necrosis)