

Given Before A.S. H.C. May 30, 1935

**CORTICAL ADRENAL EXTRACT I: A STUDY OF THE
EFFECT OF THE SWINGLE AND PFIFFNER EXTRACT ON
GUINEA PIGS INOCULATED WITH MEASURED DOSES OF
TUBERCLE BACILLI***

The fact that the medullary hormone of the adrenal quickly neutralized the effect of insulin overdosage, led us, while analyzing our 1931-32 records of insulin therapy in our tuberculous patients, to ask whether or not a physiologic imbalance in the adrenals might not help explain the marked sensitivity to insulin which was shown by some of these patients. Since most of this group were low in bodily vigor, and showed poor resistance to the disease, we felt that a study of the effect of the hormones from the adrenal bodies might be instructive.

An extract of the adrenals of cattle was made according to the method of Swingle and Pfiffner⁽¹⁾ and tested on adrenalectomized cats. It proved to be potent and was used in our work. It was not only tested in the clinic but experiments were also carried out on guinea pigs to see if any effect on the tuberculous process could be noted either during the clinical course of the disease or at autopsy.

The experimental animals lived under natural out of door conditions and were cared for as follows: Each cavy was housed in a separate cage. Each cage was placed in a long open shed and though protected from the rain and direct rays of the sun, they were exposed to normal variations of temperature. At the extremes of temperature con-

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siderable animal loss was suffered not only among the tuberculous groups but the controls as well.

Each inoculated guinea pig received 100 bacilli \pm 10 prepared from the sputum of a patient. The bacilli were given by intra-peritoneal injection.

All animals were autopsied by number, the report being filed before treatment was revealed to the pathologist. In each instance, besides the gross examination, the tissues were studied for tubercle bacilli. In all cases of rare findings the technique employed for the detection of tubercle bacilli was the dilution flotation method⁽²⁾. Weighed amounts of lymph nodes and organs were ground in mortars until quite dry. The dry tissue was then rubbed up with Sodium Hydrate, 0.5 per cent, so that the amount used was about 8 to 10 times the amount of the original tissue. After digestion at 55° for one hour, distilled water was added to the extent that one part of original material is diluted 500 times for the more vascular tissues and 300 times for the fibrous tissues, nodes and lung tissue. Xylol was then added and the material shaken in a mechanical shaker. The prepared slide was searched 20 minutes for tubercle bacilli before a negative report was made⁽³⁾. Part of the tissues were likewise examined in microscopic section by Dr. Alvin G. Foord, pathologist at the Pasadena Hospital.

The animals were observed clinically for the appearance of enlargement of the lumbo-sacral lymph nodes, the weight curves and general physical state of the animals, and the involvement of the testicles in the males.

At death the condition of the animals was designated by the state of nutrition as shown, thus:

- 0 Total absence of fat
- + Slight Omental and retroperitoneal fat
- ++ Moderate fat in abdominal structures
- +++ Sufficient fat to interfere with examination

The degree of infection was based on the weight of the spleen and the extent of involvement measured by the criteria below:

- 0 No infection
- + Less than 10 nodules in the abdomen
- ++ Moderate abdominal involvement with not more than minor thoracic involvement.
- +++ Moderate thoracic involvement with more or less extensive abdominal involvement.
- ++++ Extensive involvement of both abdominal and thoracic cavities.

Series I. Started February 14, 1933.

This experiment was devised to see what effect, if any, raw adrenal gland, cortical hormone, insulin and combined cortical hormone and insulin therapy would have on the longevity, the clinical course and pathological findings of guinea pigs. The pigs were inoculated as described above with 100 ± 10 bacilli recovered from a patient suffering from tuberculosis, and then were treated as follows:

- 6 normal guinea pigs uninoculated and untreated used as controls.
- 5 guinea pigs inoculated with 100 ± 10 tubercle bacilli, but not treated.
- 5 guinea pigs inoculated with 100 ± 10 tubercle bacilli, receiving semi-weekly feeding of 1 gram of raw adrenal gland.
- 5 guinea pigs not inoculated, receiving daily injections of $1/5$ unit (clinical unit) of insulin.*
- 5 guinea pigs inoculated with 100 ± 10 tubercle bacilli, receiving $1/50$ unit insulin daily till death
- 5 guinea pigs inoculated with 100 ± 10 tubercle bacilli, receiving $1/150$ cc. of Batch H** cortical adrenal hormone and $1/5$ unit of insulin daily till death.
- 5 guinea pigs inoculated with 100 ± 10 tubercle bacilli, receiving $1/150$ cc. Batch H cortical adrenal hormone daily till death.

All the animals were inoculated the day the treatment was started.

In analyzing the appearance of the enlarged lumbo-sacral lymph nodes as palpated through the abdominal wall, the control groups showed none. The inoculated control group showed the first palpable node during the second week and the last pig of that series during the 4th week, the average time of appearance of nodes being $2 \frac{3}{5}$ weeks. Those guinea pigs treated by the raw adrenal gland began to show involvement the second week, but not all were involved until the 4th week, the time of appearance of nodes averaging 4 weeks. In the insulin treated inoculated pigs the first nodules appeared during the third week, while in the last pig they did not appear until the 31st week. The average time

*The insulin used in this work was donated by Mulford & Company

**The cortical extract Batch H represents 37 gms. of whole raw adrenal gland to the cubic centimeter of finished product made in our laboratory according to a modification of the method of Swingle and Pfiffner.

of appearance being 13 weeks. All insulin and hormone treated animals developed their nodules between the 3rd and 6th week, averaging 4 weeks. In the adrenal hormone treated animals, 2 pigs of the 5 were negative throughout and three developed palpable nodes between the 12th and 14th week.

The development of testicular lesions in the male animals was as follows: There was no infection in either the weight control group or insulin control group. In the control inoculated group of 5 male guinea pigs, 2 pigs showed no involvement either gross or at autopsy, and the other 3 developed lesions between the 8th and 19th week, averaging the 13th week. In the raw gland group, consisting of 4 males and 1 female, testicular involvement appeared from the 9th to the 15th week, the average being the 12th week. In the insulin treated inoculated group, consisting of 4 males and 1 female, the lesions appeared between the 8th and 19th week, averaging the 23rd week. In the insulin and hormone group, 4 males and 1 female, the appearance was noted between the 11th and 17th week; and 1 was negative clinically, but at autopsy showed 2 solitary tubercles on the right testicle. In the three pigs which were positive clinically, the average appearance of the lesions was in the 14th week. In the hormone treated group of 4 males, and 1 female, 2 males were negative and 2 developed clinical lesions, during the 14th and 16th week respectively.

In analyzing the longevity of the animals, the average life of the insulin treated animals was greatest, being 279 days, or more than twice the length of life of the untreated inoculated controls which was 130 days. The raw gland and cortical hormone treated animals lived an

average of 178 and 183 days respectively. The insulin-hormone treated animals lived but 16 days longer than the unprotected animals, or 146 days.

The average weight of the spleens in grams of the untreated inoculated control animals was 14.15 grams; of the insulin, inoculated animals, 6.75 grams; of the cortical hormone treated inoculated animals, 3.09 grams. The two guinea pigs which were negative for clinical tuberculosis showed a spleen weight of .71 grams and .43 grams while the three positive pigs averaged 4.8 grams. The weight of the spleens in the insulin and cortical-adrenal hormone treated animals was 2.68 grams. The uninoculated controls averaged .84 grams and the uninoculated, insulin treated, .82 grams. The last two groups are incomplete as some of the controls are still alive.

The general nutrition as shown by the extent of fat, was almost the same for all inoculated groups, except for the insulin-hormone group which was 15+ compared with 7+ for the control inoculated group and 8+ for the other inoculated groups.

The degree of infection in all groups was nearly the same except that in the hormone treated group, 2 animals showed no infection. The other 3 pigs of this group showed lesions almost as extensive as any of the other groups.

Although the extent of lesions in all groups except the hormone treated was about the same, there seemed to be a slight difference in character. The inoculated control animals presented enormous spleens, with lesions predominantly caseous. The insulin-hormone treated group showed glandular involvement, but little infection in other structures.

SERIES I. Started February 14, 1933. 36 guinea pigs.

Number of Animals		Life	Spleen Weight	Fat	Type of Lesion	Inoculation Group	Average Last Weight	Inoculated	Positive
6	Normal animals	①	.84 gm.	④ 15	0	0	0	✓	0
5	Inoculated control	150	14.15 gm.	7	Caseous	16+	531	5	5
5	Animals treated 1 gm. raw gland semi-weekly	178	2.15 gm.	8	Fibro-caseous	16+	484	5	5
5	Uninoculated animals, 1/5 U. insulin daily	②	.82 gm	③	0	0	0	0	0
5	Inoculated animals, 1/5 U. insulin daily	279	6.75 gm.	8	Fibrous ⑤	17+	553	5	5
5	Inoculated animals, 1/5 U. insulin and 1/150 cc. Batch H, cortical hormone daily ⑦	146	2.68 gm.	15	Glandular ⑥	16+	615	5	5
5	Inoculated, 1/150 cc. Batch H, cortical hormone daily	183	3.09 gm. ⑤	8	Fibrous	9+	453	5	5

- ① 1 pig still alive. 5 dead pigs averaged 485 days.
- ② 2 pigs still alive. 3 dead pigs averaged 304 days.
- ③ 3 infected pigs averaged 4.4 gm. Uninfected 0.67 gm.
- ④ On basis of pigs dead
- ⑤ 1 pig very marked asthma
- ⑥ Huge sternal and bronchial glands with much less organ involvement
- ⑦ 1 cc. equivalent to 37 gm. of whole raw gland.

Both those treated with insulin and cortical hormone alone showed organic lesions which were predominantly proliferative. In the raw gland group the fibrocaseous type predominated.

At autopsy, the insulin-hormone treated group showed the least loss of weight. The untreated and the insulin treated inoculated group showed slightly greater loss and the raw gland and cortical hormone treated animals showed the greatest loss of all.

Series II. Started Sept. 18, 1933.

Though now underway over 2½ years, this experiment is not completed, due to survival of some of the animals. Also unfortunate weather conditions necessitated the discarding of the record of 5 animals that lived but 10 and 16 days, these being killed by extreme heat, 107°, before a sufficient period of time had elapsed following inoculation to determine whether or not infection had taken place.

In this series the animals were divided into 4 groups of 10 each.

10 normal pigs were used for weight study.

10 pigs were inoculated but untreated.

10 pigs were uninoculated but received daily injections of cortical adrenal extract Batch H (1/100 cc. daily) for 265 days.

10 pigs were inoculated and received daily injections of cortical adrenal extract Batch H (1/100 cc.) for 265 days.

The results of this experiment are as follows:

SERIES II. Started Sept. 18, 1933. 40 guinea pigs
 Inoculation: sputum #52627; 100 10 bacilli
 intraperitoneally.
 Therapy: 250 days, 1/100 cc. Batch H, Cortical
 adrenal extract.

Numbers of Animals		Life	Spleen Weight in Grams	Fat	Lesion	Inoculated	Infected
10	Normal no treatment	(4 dead 1 dead** 5 alive** 584 days 15 days 2 yr. 8mo	0.52	2.5 2	0	0	0
10	Inoculated no treatment	(8 dead 1 dead** 1 alive** 315 days 10 days 2 yr. 8 mo.	2.06 .6	1.4	3.15	8	8 Insufficient time Infected clinically
	Normal hormone treated	(6 dead 1 dead** 3 alive** 583 days 15 days 2 yr. 8 mo.	.73 .25	2.5 2	0 0	0 0	0 0
10	Inoculated hormone treated*	(6 dead 2 dead 2 dead** 318 days 786 days 15.5 days	4.44 1.1 0.5	1.8 2.5 2	2.33 0 0	6 2 2	6 0 Insufficient time

* One pig was negative grossly and only bacillus was found in pooled glands subjected to dilution flotation technique.

** Not counted in results.

Five control pigs are still living. One animal died on the 15th days from extreme heat and was not counted. The remaining 4 averaged 584 days of life; the spleens averaged .52 gms. and the fat 2.3+ at death.

Of the inoculated control group one animal died on the 10th day from heat. (Because of insufficient time for infection this animal must be thrown out.) A second animal is still alive although it has already shown definite clinical evidence of disease. The 8 animals now dead showed an average life of 313 days; average spleen weight 2.06 grams; fat average 1.4+ and a 3.13+ infection.

The cortical hormone-uninoculated group showed 1 animal that died on the 15th day (not considered in the summary); 3 animals still alive and 6 dead. Those dead, lived on an average of 583 days after treatment began; had a spleen weight of .73 grams and a 2.5+ average fat at death.

In the hormone treated inoculated group 2 pigs died on the 15th and 16th days. These were negative on examination, but must not be considered because of insufficient time for infection. Of the remaining 8, 2 failed to develop any evidence of the disease. A third was negative by gross examination, yet by subjecting the pooled glands to maceration and the dilution flotation technique, 1 bacillus was found in a ten-minute search.

The 6 pigs infected showed an average length of life of 318 days; 4.44 grams spleen average; 1.8+ fat average; 2.53+ infection. The negative animals showed 1.1 gram spleen average; 2.5+ fat and no lesion.

Series III, Started June 5, 1934.

In this series, 50 guinea pigs were studied, being divided into groups of ten. All animals received 1/10 cc. of #1 P.P.D tuberculin before inoculation. All were negative. The purpose of this experiment was to see if there was any difference in the effect which could be traced to the time when the cortical therapy was administered, and to further study the suggested protective action against infection.

The guinea pigs were grouped as follows:

- 10 normal pigs as controls
- 10 pigs inoculated but untreated
- 10 pigs given 10 days of 1/150 cc. Eschatin* before inoculation
- 10 pigs given 10 days of 1/150 cc. of Eschatin* before and after inoculation
- 10 pigs given 10 days of 1/150 cc. Eschatin* after inoculation.

This experiment was not designed to be a longevity test, but was to be terminated at the close of three months. Unfortunately, the groups were badly riddled by an unprecedented heat spell which gave us several days of extreme heat during the short space of the experiment. Fortunately, however, the first casualties occurred 36 days after inoculation, so that the infection was well under way. Although the weight curves and group comparisons are not satisfactory on this account the apparent protection of 8 pigs out of 30 seems significant.

All inoculated control animals were infected. Their total infection was rated a 21+. The animals that had 10 days of hormone

*Part of the Eschatin for this work was furnished by Parke, Davis & Company.

SERIES III. 50 animals. Therapy: 1/150 cc. Parke-Davis Eschatin

<i>Number of Animals</i>		^① <i>Total Life</i>	<i>Inoculated</i>	<i>Infected</i>	<i>Spleen Weight in grams</i>	<i>Last Weight Group Average</i>	<i>Extent of Lesion</i>
10	Weight control	640	0	0	.85	564	0
10	Inoculated control	690	10	10	1.06	578	21
10	Hormones 10 days before inoculation	956	10	8	.92	626	13
10	Hormones 10 days before and after inoculation	703	10	6	.88	566	12
10	Hormones 10 days after inoculation	579	10	8	.79	539	15

① Maximum possible group life, 980 days.

therapy before and 10 days after inoculation showed but 12+. The group receiving 10 days therapy before inoculation showed only 13+, and the group receiving therapy for 10 days after inoculation had 15+. This showed practically the same effect regardless of the time of administering the hormone.

The spleen weight for the normal animals averaged .85 grams. The inoculated control group showed 1.06 grams. Those having 10 days preliminary treatment showed an average of .92 grams. The spleens of the animals receiving 10 days treatment before and after averaged .82 grams, and those receiving treatment 10 days after inoculation, .79 grams.

These results are interesting in connection with Steinbach's experiments. Steinbach⁽⁴⁾ rendered immune rats susceptible to bovine tuberculosis by adrenalectomy and also increased their susceptibility to avian tuberculosis. The accumulated literature on the value of the suprarenals in infectious diseases is beyond the scope of this report. We are continuing our studies and hope to make a report on a larger group of animals in the near future.

We have made another observation which may be of considerable importance but which we desire only to mention at this time, because it has a bearing upon the phenomenon of immunity. In treating asthmatic children with potent cortical extract, we have seen 7 children who showed positive reaction to No. 1 P.P.D. tuberculin at the beginning of treatment become negative to a stronger tuberculin No. 2, P.P.D. after a period of treatment of from six months to one year.

SUMMARY

Summarizing the above groups and comparing them with normals, we find that in the adrenal cortex treated animals, the average weight of the spleens of 20 normal animals was .79 grams. The average weight of spleens in 23 untreated inoculated animals was 3.97 grams. The 6 hormone treated uninoculated animals had an average of .73 grams and the 43 hormone inoculated animals, 1.69 grams.

The two uninoculated groups showed no infection, while 23 of 23 controls were infected, as well as 15 out of 15 animals in the other groups of the first series. But 12 of the 43 hormone treated, inoculated animals did not develop tuberculous disease when inoculated with virulent bacilli.

It is possible that a more careful study of dosage and administration might render protection to a greater percentage of animals.

F. M. Pottenger, Jr. and
J. E. Pottenger

From the Research Department
Pottenger Sanatorium
Monrovia, California

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Cortical Adrenal Extract I: A study of the effect of the Swingle and Pfiffner extract, or guinea pigs inoculated with measured doses of Tubercle bacilli
Insulin and adrenal extract,
Pfiffner and Swingle and test on cat.

Care of experimental animals.
Temperature.

Inoculation, 100-10

Autopsy by no.

Study by no.
Gross and microscopic
Dilutatio flotation method

NaOH 5% 8-10.
3-500 dil.
Xylol and shaker.

Search 20 minutes
Alvin Foord

Clinical examination,
Nodes, testicles,
weight and physical state.

Slides: Fat
Infection
Series I
Series II
Series II weight
Series III

Steinbach and rats

Slide summary

Possible study of dosage.