mesenteries and intestines. With amounts of 15 mg or less in about 1.0 cc the animals, while at first becoming sick, eventually recovered; no residua were noted on autopsy several days later. Because of the acute toxicity of the drug when administered in this fashion a series was run in which the free acid was placed either subcutaneously through an incision or injected as an emulsion in about 1.0 cc of water in the loose tissues about the neck. Amounts below 30 mg per 30 g mouse were fairly well tolerated; the main reaction again was an accumulation of sterile gelatinous exudate which was later resorbed.

A known virulent C-203 strain was injected subcutaneously along side about 15 mg of *o*-iodosobenzoic acid and at no time was there evidence of infection in the 6 animals used; the controls without *o*-iodosobenzoic acid all died within 48 hr. In the presence of the same amount of drug as many as 5×10^7 organisms had no more effect than 1×10^3 and all cultures from the site of the injections and the heart's blood failed to show hemolytic streptococci. In a series of animals receiving 60 mg of the drug all were dead within 48 hr as a result of the toxic effects of the drug but none showed any signs of infection on autopsy or culture.

Conclusions. 1. *o*-iodosobenzoate *in vitro* apparently has as marked if not more marked an effect than the sulfonamides on *E. coli* and certain hemolytic streptococci. 2. The effect is bactericidal rather than bacteriostatic. 3. It is conceivable that the drug may act by interruption of some catalytic process dependent on intact —SH groups. 4. Locally *o*-iodosobenzoic acid apparently did control in these experiments what should have been an overwhelming infection. The *o*-iodosobenzoate which is much more soluble seems to be too toxic to be used locally.

These data suggest that *o*-iodosobenzoic acid may be worth further study as a possible bactericidal agent in the treatment of infected wounds.

It is a pleasure to thank Dr. Charles L. Fox, Jr., for many valuable suggestions in the bacteriological technics involved.

13956

Injurious Action of Pitressin on the Rat Testis.*

MIRIAM E. SIMPSON, HERBERT M. EVANS AND CHOH HAO LI.

From the Institute of Experimental Biology, University of California, Berkeley, California.

Some of the biological effects of adrenocorticotropic pituitary preparations suggested the presence of a pressor substance. A temporary prostration of the rats was observed invariably to follow injections of high levels of these partially purified preparations. Coincidentally it was noted that there were injurious effects of high levels of these preparations on the testes of normal and hypophysectomized males. Two methods have been employed in the effort to determine whether the injurious effects on the testes may be referred to pressor principles. First, attempts were made to remove any pressor substance from ACT preparations and to compare the biological properties of the crude and purer materials. This phase of the subject will be discussed later. Second, effects of the pressor principle itself on the testis have been studied and are here reported.

As a maximum of 5 dog pressor units per mg have been found in ACT preparations, pitressin† was injected into normal 40-day-

* Aided by grants from the Research Board of the University of California, the Rockefeller Foundation, New York City, and Parke, Davis & Company, Detroit. Assistance was rendered by the Work Projects Administration, Official Project No. 265-1-08-80, Unit A-5.

† Kindly supplied by Parke, Davis and Co.

TABLE I.
Effects of 5 Pressor Units (Dog) Daily of Pitressin on Reproductive System of Normal Immature Male Rats.

Treatment	Age at onset, days	No. of rats	Injection period, days*	Organ wt‡		
				Testes, mg	Seminal vesicles, mg	Prostate, mg
Pitressin	26	5	23	1248	48	189
Control at onset, 25 da	—	5	—	284	11	52
Control at autopsy, 49 da	—	7	—	1734	99	211
Pitressin	40	5	15	1458	161	271
Control at onset, 41-44 da	—	58	—	1206	41	132
Control at autopsy, 55-56 da	—	36	—	2305	231	282

*Injections were intraperitoneal and were given daily except Sunday, hence 13 injections were given in the 15-day period, 18 injections in the 23-day period.

old male rats for a period of 15 days, at a dose of 5 pressor units daily. As can be seen in Table I, the testis was definitely decreased in weight by this treatment—almost halved.‡ Spermatozoa were absent in the epididymis, or were present in small numbers only, whereas the epididymis of the normal controls (55 days old) contained abundant spermatozoa. Histologically, the testes showed varying degrees of injury. The normal testis of the rat at this age contains tubules of uniform caliber, which have not yet attained their maximum diameter, and mature spermatozoa or spermatids are found in practically all tubules. The interstitial cells have acquired a considerable body of cytoplasm and are epithelioid rather than "connective tissue like." After pitressin treatment, the tubules were usually lined by spermatocytes; sometimes spermatids were present and occasionally a few abnormal spermatozoa. A variable number of tubules, in some cases most tubules, showed not only retardation of development but marked evidences of injury. Some showed sloughing of the entire epithelial investiture down to the basement membrane, and in a few instances complete disintegration of tubules occurred. The interstitial tissue, though normal in appearance, did not seem to be functioning at a normal rate, judged by the weight of the accessories. Development of the seminal vesicles was definitely retarded, that of the prostate only slightly.

‡ Body weight increase was also retarded by about 20 g. (Body weights were the same at onset, the normals gained 80 g, the injected 64 g.)

As can be seen in Table I, similar results were obtained in 26-day-old normal males injected daily for 23 days with 5 pressor units.

The injurious effects of pitressin on the testis were also demonstrable in the hypophysectomized rat. Testis weights were smaller in hypophysectomized rats receiving either 1.0 or 0.1 units of pitressin daily. Histological examination made clear that this decrease was due to injurious effects on the tubules. The testis of the rat hypophysectomized at 40 days of age when allowed to regress without treatment for 15 days decreases in weight far below that characteristic at the time of operation. Tubules become uniformly smaller; spermatids disappear; the number of layers of spermatocytes is reduced, although some healthy, adherent spermatocytes of the first order are still present in most tubules after this postoperative interval. By this time also, characteristic changes in the chromatin of the Leydig cells have occurred. The testes of hypophysectomized rats which had received pitressin from the time of operation did not show this orderly picture. Superimposed upon the regular regressive changes were obvious signs of injury, such as death and extensive desquamation of tubular epithelium. Interstitial cells were not further affected, nor was the usual regression of seminal vesicles and prostate influenced. (Table II).

The behavior of animals receiving pitressin

TABLE II.
Effect of Pitressin Injected from Time of Operation on Regressing Reproductive System of Male Rats Hypophysectomized at 40 Days of Age.

Treatment	No. of rats	Daily dose* (IP)	Injection period, days	Organ wt		
				Testes, mg	Seminal vesicles, mg	Prostate, mg
Pitressin	4	1.0	15	360	16	47
"	4	0.1	15	322	14	42
Hypophysectomized controls, 55 da old	32	—	—	431	17	51

*Dog pressor units.

was observed to see if the prostration effect of ACT preparations was given by the doses of pitressin which injured the testes. The 5 pressor unit dose, corresponding to the amount of contamination in the usual dose of ACT, 1 mg, had a corresponding effect on the animal. The drowsy appearance, labored breathing and slowed righting reflex lasted for 10-20 min after each injection; 45 min after injection the animal appeared entirely normal.

Conclusion. Pitressin in high dosage has been found to be injurious to the tubular epithelium of the testis of the rat. In interpretation of the biological effects of extracts of pituitary substance it is therefore extremely important to keep in mind the possibility of this contaminant.

13957

The Attenuating Effect of Promin on Virulence of the Tubercle Bacillus.

E. W. EMMART AND M. I. SMITH.

From the Division of Chemotherapy, National Institute of Health, U. S. Public Health Service, Bethesda, Md.

It has been shown previously that p,p'-diaminodiphenylsulfone-N-N'-dextrose sulfonate (promin) in concentrations of 20 mg % inhibits the growth of the tubercle bacillus in culture media.[1] Also promin administered orally[1] or in the diet[2] retards the tuberculous process in guinea pigs. To determine the mechanism of the action of promin upon the virulence of the tubercle bacillus experiments were designed to ascertain the effect of prolonged cultivation of the bacillus on the medium containing the drug in low concentrations. Changes in virulence of the strain were determined by the degree of invasiveness of the attenuated strain, (a) when inoculated upon the chorio-allantoic membrane of the chick embryo and (b) when inoculated intraperitoneally into guinea pigs.

Methods. The A 27 strain of human tubercle bacilli was selected since this strain was known to have a moderately high degree of virulence. The bacilli were grown in a culture medium consisting of beef bouillon plus 5% glycerine with 10 mg % of promin. Preliminary experiments had shown that the strain could not survive this concentration of promin continuously; hence, after the second transfer into the medium containing promin, the strain was returned to the control medium. After the strain had recovered its capacity to grow in the control medium, it was again placed in the medium containing promin. Finally, the suspension for inoculation was made from bacilli grown in the

1 Smith, M. I., Emmart, E. W., and Westfall, B. B., *J. Pharm. and Exp. Ther.*, 1942, **74**, 163.

2 Feldman, W. H., Hinshaw, H. C., and Moses, H. E., *Am. Rev. Tuberculosis*, 1942, **45**, 303.