

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.

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

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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 orally¹ or in the diet² 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 in-

oculated 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.

promin-free culture medium. The total attenuation period with promin in the divers experiments varied from 24 hr to 104 days, comprising 1 to 4 transfers in promin and 2 to 3 in control medium. The ultimate decrease in the virulence of the strain may be considered, therefore, a result of the accumulative effect of the drug after prolonged treatment.

In all experiments bacillary suspensions were prepared by weighing the bacilli and diluting to the desired concentration with normal saline so that .2 cc contained 1 mg of bacilli. Finely dispersed suspensions were obtained by rotating the weighed bacilli in a White Ball Bacteria Grinder for 30 min. Each egg received 1 mg of bacilli on the outer surface of the chorio-allantoic membrane. The technic of inoculation of the membranes, their fixation, the embedding and staining, and evaluation of the extent and degree of tuberculous involvement was the same as previously described.³ The average extent of tubercle formation in all membranes of each experiment was compared with a similar group inoculated with bacilli

of the same strain cultured in control medium. The differences indicate the extent of attenuation achieved.

As an additional check on the relative virulence of the control strain and the promin-treated strain of tubercle bacilli, 2 groups consisting of 15 guinea pigs each and weighing on the average 350 g were inoculated intraperitoneally with 0.5 mg tubercle bacilli, the one with a suspension prepared from the strain of bacilli which had been treated with promin for 104 days and the other with a suspension of untreated bacilli. The animals were weighed once a week. This experiment was terminated after 50 days when all survivors were killed and autopsied. The extent of tuberculous involvement in the omentum and glands, spleen, liver, lungs, peritoneum and kidneys was noted and the "tuberculosis index" evaluated as previously described.³

Results. A. Chick Embryo Technic. Table I summarizes the results of the experiments with the chorio-allantoic membrane. With the exception of experiments 59 and 69 the promin-treated bacilli were returned to a normal medium before preparing the suspension for inoculation. In experiments 59 and

TABLE I.
Effect of Attenuation of Virulence of Tubercle Bacilli of the A 27 Strain After Treatment with Promin.

Exp. No.	No. of chick embryos inoculated	No. of embryos surviving	Total days of strain of bacilli in promin media	Total days of strain in control media	% macroscopic tubercles	Microscopic observations Avg degree of cellular proliferation and aggregation, evaluated on basis of 0 to 4			
						Epi- thelium	Eosino- phils	Monocytes	Tubercle formation
I. Chorio-Allantoic Membranes Inoculated with Promin-Treated Bacilli.									
59	27	13	1	0	57	1.0	1.0	1.4	0.4
69	30	8	1	0	41	0.6	1.6	1.5	0.6
52	25	8	22	90	75	1.0	0.8	1.1	0.8
49	27	9	54	31	66	2.0	1.3	1.7	1.3
50	24	5	54	52	50	1.0	1.5	1.2	0.7
53	24	8	54	65	100	2.3	2.0	3.0	2.6
54	22	5	54	64	100	2.0	2.2	3.0	2.8
74	24	21	104	103	80	1.3	1.3	1.7	1.3
Avg						—	—	—	—
						1.4	1.4	1.8	1.3
II. Chorio-Allantoic Membrane Inoculated with Bacilli from Control Media.									
22	33	8	0	Continuously	100	2.2	2.6	3.6	3.8
51	24	18	0	"	95	1.6	1.7	2.2	1.9
55	18	4	0	"	100	1.0	2.0	2.2	2.0
70	36	18	0	"	100	1.5	2.1	2.5	2.1
75	24	19	0	"	100	1.8	2.4	3.0	3.0
Avg						—	—	—	—
						1.6	2.1	2.7	2.5

³ Emmart, E. W., and Smith, M. I., *Am. Rev. Tuberculosis*, in press.

TABLE II.
A. Comparison of Degree of Tubercle Development in Membranes Inoculated with Promin-Treated Tubercle Bacilli and with Bacilli Grown on the Control Medium.
— None, + Slight, ++ Moderate, +++ Advanced, ++++ Extensive.

Embryo No.	Macroscopic tubercles	Degree of microscopic cellular proliferation and aggregation			
		Epithelium	Eosinophils	Monocytes	Tubercle development
I. Chorio-Allantoic Membranes Inoculated with Tubercle Bacilli Cultured for 104 Days on Media Containing Promin—Exp. 74.					
1	+	+++	+	+	+
2	+	+++	+++	++	++++
3	+	—	—	—	+
4	+	++	+++	+++	++
5	+	+	++	+++	+
6	+	+	++	+++	++
8	+++	++	+++	++++	+++
9	+	+	—	+	+
11	+	+	+	++	+
12	+	+	—	+	+
14	—	+	+	+	—
15	+	+	+	+	+
16	+	+	+	+	+
18	—	+	—	+	+
19	+++	++	+++	++++	+++
20	—	+	+	+	+
22	+	+	++	++	+
23	+	++	+	+	+
24	—	+	+	+	—
Avg	1.0	1.3	1.3	1.7	1.3
II. Chorio-Allantoic Membranes Inoculated with Tubercle Bacilli from Control Cultures—Exp. 75.					
2	++++	++	+++	++++	++++
3	++++	+	++	++++	++++
4	+++	+	++	+++	++
5	+	+	+	+	+
6	++++	+++	+++	++++	++++
9	++++	+++	+++	++++	++++
10	+++	++++	+++	+++	++++
14	++	+	++	+++	++
15	+++	++	+++	++++	+++
16	+	+	+	+	+
17	++++	++	+++	++++	++++
18	++++	++	++++	++++	++++
20	++	+	+	+	+
21	++++	++	++++	++++	++++
22	+	—	—	—	+
23	++++	+++	++++	++++	++++
Avg	3.0	1.8	2.4	3.0	3.0

69 the suspension was prepared from tubercle bacilli grown on a normal medium but subjected to 10 mg % promin for 24 hr at 37°C before inoculation. In these 2 experiments with bacilli treated with promin 24 hr, the surviving eggs had an average of 49% tubercle formation. Usually in 6 days membranes inoculated with 1 mg of normal control A 27 bacilli had 100% gross tubercles. The results from experiments 59 and 69 represent tuberculocidal action rather than attenuation

which probably explains the low incidence of tubercle formation in these 2 experiments.

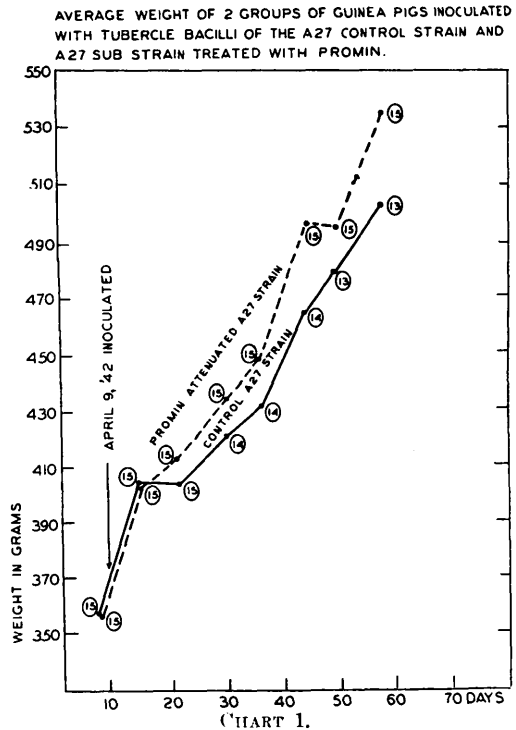
One attenuation experiment carried for 22 days in promin and 90 days in control resulted in 75% tubercle formation. Other cultures carried 54 days in promin and for increasing lengths of time in control medium varied from 50 to 100% in the production of tubercles. The tubercles, however, were usually small, fibrous and often not more than several aggregates of epithelioid cells.

Of the surviving embryos the membranes inoculated with promin-treated bacilli 74% were either negative or possessed tubercles in early stages of development, while only 26% showed large tubercles. In the control strain with an incidence of nearly 100% tubercle formation in almost every instance the tubercles were large, fully developed and showed caseation and necrosis. The difference in the virulence of the promin-treated strain and the control strain was well illustrated after 104 days of treatment with promin. This strain after culturing in control medium for 29 days was suspended in normal saline and implanted on the membranes of 24 embryos. Fifty-eight per cent of the membranes so inoculated showed a slight tubercle development, 12% extensive tubercle development and 8% none at all. In the control experiments all membranes had tubercles; of these, 75% showed extensive tubercle development and 25% slight tubercle development. The data of this experiment are given in detail in Table II.

Microscopic examination of the sections of the chorio-allantoic membranes revealed marked differences in the virulence of the treated and non-treated strain of bacilli. The degree of epithelial proliferation, the number and extent of the aggregations of eosinophils and monocytes and the degree of tubercle development was generally less pronounced in the membranes implanted with promin-treated bacilli.

The average incidence of tubercle formation in the 2 series of experiments was nearly twice as high in the controls as compared with the promin-treated strain (Table I) and nearly 3 times as high when a comparison is made on the basis of tubercle formation on individual membranes implanted with bacilli treated with promin for 104 days (Table II). Differences in the several types of cellular proliferation were not the same in both series of experiments. Generally the differences in the extent of proliferation of the surface epithelium were least marked while those of monocytic proliferation were the most pronounced.

B. Guinea Pig Experiments. As an additional check on the relative difference in the



virulence of the promin-treated strain of bacilli as compared with a control strain, a suspension prepared from a strain of bacilli treated 104 days with promin and one from a control strain were inoculated into 2 series of guinea pigs. Chart 1 shows graphically the differences in weights of these 2 series of guinea pigs. Two weeks after the inoculations the series of guinea pigs inoculated with the control strain lost weight more rapidly than the animals inoculated with the promin attenuated strain. This difference was maintained throughout the experiment. Fifty days after inoculation, when the experiment was terminated, the difference between the average weights of the guinea pigs inoculated with the promin-treated strain and the control strain was 37 g. The divergence of the 2 curves was accentuated as the animals in the control group developed symptoms of the more advanced stages of the disease.

In the series of animals inoculated with promin-treated bacilli none died within the experimental period. In the control group 1 died on the 23rd day and another on the

42nd day, and the rest were killed at the termination of the experiment. Upon autopsy, the degree of tuberculous involvement in the lungs, omentum, liver, spleen, and peritoneum and kidneys was evaluated on the basis of a possible maximum of 20 as previously described.³ The control group showed a tuberculosis index of 9.0 as compared with an index of 6.6 for the group inoculated with promin-treated bacilli. From the differences in the weight curves as well as from the tuberculosis index of the autopsied animals it appears that the results of inoculation of the treated and untreated strains into guinea pigs parallel the results obtained when the same strains were implanted on the chorio-allantoic membrane of the chick embryo.

Conclusions. 1. Experiments are described to show that prolonged cultivation of tubercle bacilli on a promin-containing medium attenuates their virulence. 2. Under the experimental conditions the reduction in virulence in the promin-treated strain persisted after the strain was returned to control media. 3. The change in virulence can be demonstrated both by implantation on the chorio-allantoic membrane of the chick embryo as well as by inoculation into guinea pigs. 4. The attenuating action of promin is slight, this being probably related to the relatively low bacteriostatic action of this drug against the tubercle bacillus. It seems likely that more promising results might be obtained by the application of this method to more active bacteriostatic agents.

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Action of Alkaline Acriflavine Solution on *Bacterium salmonicida* and Trout Eggs.*

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Trout furunculosis is a costly, widespread, epizootic bacteremia, that annually destroys millions of salmon and trout.¹ The disease occurs in trout hatcheries and rivers in at least 8 European countries, in Canada, and in more than one-fourth of the United States. Trout furunculosis is caused by *Bacterium salmonicida* Lehmann and Neumann, a gram-negative non-spore-forming rod which shows deeply-staining, bipolar bodies; the taxonomic position of this bacterium has not been determined.

Among the agencies by which furunculosis may be disseminated are trout ova, which in

practical fish culture are freely transported from contaminated hatcheries and spawning beds to clean hatcheries. In fish hatcheries, eggs are usually incubated in headwaters; and thus may be a source of infection to fish downstream. The interior of living trout eggs is believed to be bacteriologically sterile.^{2,3} Only the dead eggs and the exterior of living ones can be considered as sources of infection. The former may readily be removed manually. If, therefore, the exterior of the living ova can be disinfected, eggs may be placed in headwaters in hatcheries, without danger to fish downstream.

* Subsidized by and carried out in the laboratories of the Wisconsin Conservation Department. Technical assistance contributed by the Fisheries Research Project, Work Projects Administration, Madison, Wisconsin.

¹ Mackie, T. J., and Menzies, W. J. M., *J. Comp. Path. and Ther.*, 1938, **51**, 225.

² Plehn, M., *Centl. Bakt. (etc.)*, Abt. Orig., 1911, **60**, 609.

³ Williamson, I. J. F., *Fisheries, Scotland, Salmon Fish*, 1929, No. 1, His Majesty's Stationery Office, Edinburgh, 1929.