effect when cobalt injections are combined with the altitude exposure.

There may be 2 stages in the development of polycythemia. The first phase is characterized by a normal whole blood volume with a subnormal plasma volume. The second phase shows a normal rate of increase in plasma volume with a further increase in whole blood volume.

Subcutaneous cobalt injections slowly induce polycythemia in rabbits but they are accompanied by anorexia, sterile abscesses and other symptoms of toxic effects.

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Effect of Streptomycin on Growth of Rickettsiae in Eggs.

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In experimental infections, rickettsiae have been shown to be susceptible to the action of penicillin[1] as well as to other therapeutic agents.[2-6] In view of the increasing availability of streptomycin, it was of interest to determine its effect on the growth of representative pathogenic rickettsiae. Using the yolk sac technic of Cox,[7] the action of streptomycin on the growth of the rickettsiae of the Wilmington strain of murine typhus, the Breinl strain of epidemic typhus and the Karp strain of tsutsugamushi disease in embryonated eggs was studied and compared with the action of *p*-aminobenzoic acid (PABA).

Methods. Streptomycin dissolved in 0.85% saline was sterilized by filtration through a Seitz filter previously washed with distilled water. A sterile solution of PABA in buffered saline was also prepared. The concentration of these 2 solutions was so adjusted that the dosage employed was contained in 0.4 ml, which was inoculated into the yolk sac of 7-day-old embryonated eggs. The same volume of sterile buffered saline was inoculated into control eggs. Two hours later, both treated and control eggs were inoculated by the same route with a suspension of yolk sacs infected with the Breinl strain of *Rickettsia prowazeki* or the Wilmington strain of *R. mooseri* or the Karp strain of *R. orientalis.* The infective suspensions had been previously titrated in eggs; a dilution was selected which just permitted the survival of a majority of the embryos for 7 days. Uninfected control eggs received injections of the same amounts of streptomycin as a check on its toxicity. The activity of the streptomycin was checked by testing dilutions of the stock solution against a susceptible strain of *Escherichia coli.* Some of the eggs received a second injection of streptomycin 72 hours later.

The eggs were incubated at 35°C for 7 days and candled daily; the dead eggs were discarded. Deaths occurring before the 3rd day were considered to be nonspecific and were disregarded. On the 7th day of incubation, the yolk sacs from all survivors in each group were harvested, divided into

[1] Greiff, D., and Pinkerton, H., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 116.

[2] Snyder, J. C., Maier, J., and Anderson, C. R., Report to the Division of Medical Sciences, National Research Council, Washington, D.C., Dec. 26, 1942.

[3] Greiff, D., Pinkerton, H., and Moragues, V., *J. Exp. Med.*, 1944, **80**, 561.

[4] Hamilton, H. L., Plotz, H., and Smadel, J. E., PROC. SOC. EXP. BIOL. AND MED., 1945, **58**, 255.

[5] Hamilton, H. L., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 220.

[6] Smadel, J. E., Snyder, J. C., Jackson, E. B., Fox, J. P., and Hamilton, H. L., (Abstract), *Fed. Proc.*, 1946, **5**, 254.

[7] Cox, H. R., *Pub. Health Rep.*, 1938, **53**, 2241.

TABLE I.

Effects of Streptomycin and *p*-Aminobenzoic Acid on the Multiplication of *Rickettsia prowazeki*, *R. mooseri*, and *R. orientalis* in Embryonated Hens' Eggs.

Strain	Experiment	Drug	Dose for each egg, mg	Interval between therapy and infection, hr	Ratio surviving embryos to total in groups at 7th day	No. of viable rickettsiae per ml of yolk sac pools*
R. prowazeki (Breinl strain)	I	Streptomycin	0.5	—2	13/15	$10^{6.3}$, $10^{5.3}$
		"	0.5	—2, +72	11/15	$10^{7.9}$, $>10^{6.3}$
		PABA	5.5	—2	11/14	$<10^{1.3}$
		Saline		—2, +72	7/9	$10^{8.3}$, $10^{9.0}$
		Drug controls				
		Streptomycin	0.5	—2, +72	11/13	
	II	Streptomycin	0.5	—2	7/9	$>10^{6.3}$
		"	1.0	—2	11/12	$10^{7.8}$, $>10^{6.3}$
		"	2.0	—2	14/15	$10^{7.6}$, $>10^{6.3}$
		Saline		—2	13/15	$10^{8.6}$
	III	Streptomycin	2.0	—2	9/12	$10^{5.9}$, $10^{7.3}$
		Saline		—2	9/12	$10^{8.4}$, $10^{8.8}$
R. mooseri (Wilmington strain)	IV	Streptomycin	2.0	—2	11/13	$10^{6.2}$, $10^{6.3}$
		PABA	11.0	—2	4/8	$<10^{1.3}$
		Saline		—2	13/14	$10^{7.6}$, $10^{7.3}$
		Drug controls	2.0	—2	9/10	
R. orientalis (Karp strain)	V	Streptomycin	2.0	—2	11/13	$10^{5.3}$, $10^{5.0}$
		PABA	11.0	—2	4/8	$10^{1.7}$
		Saline		—2	12/13	$10^{5.8}$, $10^{5.6}$

* Numbers estimated from 50% end points in animal tests. See text for description of methods. When 2 figures are given, they represent values from duplicate determinations. The symbols > and < indicate that the end points fell beyond the titration dilutions employed.

2 lots each containing 3 to 7 sacs, and shaken in bottles with glass beads and an appropriate amount of sterile diluent (nutrient broth for *R. prowazeki* and *R. mooseri* and skim milk for *R. orientalis*) to make a 50% suspension and stored in sealed glass ampoules in a carbon dioxide cabinet.

Subsequently these yolk sac suspensions were thawed, lightly centrifuged, and diluted in broth or skim milk for injection into animals. The serial 10-fold dilutions of yolk sacs infected with the Breinl strain of *R. prowazeki* were inoculated intraperitoneally into 4 cotton rats for each dilution. Three weeks later, all of these rats were tested for immunity by the intracardial inoculation of approximately 4 "certainly fatal doses" of yolk sacs infected with the Breinl strain. The 50% immunizing end-point of the treated and control yolk sac pools was calculated[8] on the basis of the immunity produced in cotton rats in such challenge experiments.

The yolk sac pools infected with the Wilmington strain of *R. mooseri* were titrated by injecting 0.25 ml of the serial 10-fold dilutions in nutrient broth into each of the 8 albino mice, by the intraperitoneal route. Three weeks after injection, the surviving mice were tested for immunity by the intravenous injection of 0.2 ml of an infected yolk sac suspension containing 4 LD_{50} "toxic doses." The 50% immunizing end-points of the drug-treated and control yolk sac pools were calculated on the basis of the number of mice surviving 10 days after the challenge.

Titrations of the yolk sac pools infected with the Karp strain of *R. orientalis* were carried out by injecting 0.25 ml of the serial 10-fold dilutions prepared in skim milk intraperitoneally into albino mice using 8 mice per each dilution. The mice were observed for 21 days, and the 50% end-points were calculated on the basis of the survivors in each group of 8 mice.

For convenience in evaluating results the minimal quantity of viable rickettsiae required to produce immunity in cotton rats (*R. prowazeki* and *R. mooseri*) or death in white mice (*R. orientalis*) is assumed to be one viable organism. It is appreciated that the minimal quantity may be more than one organism, but for the purpose of comparison in these tests, this point is disregarded.

Results. The data are summarized in Table I. Streptomycin appears to have some inhibiting effect on the growth of *R. prowazeki* in embryonated eggs. The "numbers of viable rickettsiae" in eggs infected with *R. prowazeki* and injected with 0.5 mg of streptomycin were $10^{5.3}$ and $10^{6.3}$, as compared with $10^{8.3}$ and $10^{9.0}$ in the control eggs. A second injection of 0.5 mg, 72 hours after infection did not increase this effect. Increasing the amount of streptomycin to 1.0 and 2.0 mg did not appear to increase the inhibitory effect. The mean value of the numbers of *R. prowazeki* in streptomycin-treated eggs in the 3 experiments (7 titrations which properly spanned the end-points) was $10^{6.9}$ as compared with the mean value of $10^{8.6}$ attained in control eggs (5 titrations). The difference is statistically significant (Student's "t" test; P lies between 0.05 and 0.02).

In the case of *R. mooseri,* the mean value for the eggs receiving 2.0 mg of streptomycin was $10^{6.3}$ rickettsiae as compared with $10^{7.5}$ for the controls. This difference would be expected to occur by chance only once in more than 20 times.* These effects are small when compared to the striking reduction of growth of both *R. prowazeki* and *R. mooseri* with PABA.

Streptomycin in doses of 2.0 mg per egg had no significant effect on the growth of *R. orientalis* in embryonated eggs. This is demonstrated by the "numbers of viable rickettsiae" in yolk sacs from treated eggs, $10^{5.3}$ and $10^{5.0}$, as compared with $10^{5.8}$ and $10^{5.6}$ in the untreated controls. These results with 2.0 mg of streptomycin are in contrast with those obtained with 11 mg of PABA which produced a 10,000-fold decrease in the amount of growth obtained with *R. orientalis.*†

[8] Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

* The technic of titration and the reproducible nature of the results obtained with it will be discussed in detail in another publication.

Two experiments were performed to determine whether streptomycin was rapidly inactivated in the chick embryo and thus might appear to have little effect on rickettsial growth when compared with PABA (which is known to remain at adequate levels in the egg for the period of time covered by these experiments.)[9] Streptomycin does not appear to be inactivated, since allantoic fluids and yolks harvested 4 days and 7 days after the inoculation of eggs with 1.0 mg by the yolk sac route were shown to contain the expected amounts, that is, between 5 and 25 μg per ml, by assay with 3 strains of organisms susceptible to streptomycin, *E. coli* (Waksman) and *Bacillus friedländeri* (Yarsik) and *B. friedländeri* (Lundgren).

Discussion. The concentrations of streptomycin in these tests were selected to represent approximately the concentrations attainable in man. With therapeutic effects in eggs of such a slight degree, it seems unlikely that streptomycin would have any very striking effect on human infections caused by *R. prowazeki, R. mooseri,* or *R. orientalis.* The effects are sufficient, however, to caution against the use of streptomycin in the attempt to isolate *R. prowazeki* or *R. mooseri* from material contaminated with streptomycin-sensitive bacteria, particularly if the numbers of rickettsiae in the material are small.

Summary. Streptomycin in amounts of from 0.5 to 2.0 mg injected into infected embryonated eggs has a definite slight inhibitory action on the growth of *R. prowazeki.* In the case of *R. mooseri,* 2.0 mg of streptomycin had a small but significant effect. With *R. orientalis,* a dose of 2.0 mg per egg appeared to have no significant effect on the multiplication of the rickettsiae. By contrast, appropriate doses of PABA (5.5 mg per egg for *R. prowazeki,* and 11.0 mg per egg for *R. mooseri* and *R. orientalis*) produced a striking inhibition of growth, reducing the numbers of viable rickettsiae by 10,000 to over 10,000,000 times as compared with untreated controls.

Addendum. As this manuscript was in preparation, Dr. J. E. Smadel informed the authors that his experiments showed a very definite therapeutic effect of streptomycin against infection of embryonated hens' eggs with *R. prowazeki* when doses of 10 mg (10,000 units) per egg were employed, 5 times the maximum dose administered in the experiments herein reported.

† The minimum concentration of PABA required to produce a detectable inhibition of the growth of *R. orientalis* is considerably greater than that required for inhibition of *R. prowazeki.*[9]

9 Snyder, J. C., and Stevens, D. A., unpublished observations.

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Action of Curare on Temperature Changes in the Brain in Combination With Pentobarbital Narcosis.

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It has been demonstrated that curare-alkaloids (Merck strychnos curare, crystallized tubocurarine hydrochloride) and dihydroerythroidine bromide have a central action, besides the peripheral effect on the neuromuscular junction.[1,2] The central action of these alkaloids was first demonstrated on the electroencephalogram of frogs. At first the amplitude and the frequency of the action

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1 Feitelberg, S., and Pick, E. P., PROC. SOC. EXP. BIOL. AND MED., 1942, **49**, 654.

2 Pick, E. P., and Unna, K., *J. Pharm. and Exp. Therap.*, 1945, **83**, 59.