solutions 10^{-4} or 10^{-5} M, show a tendency to assume a spherical shape. Their surfaces display a considerable activity as manifested by rapid protrusion and withdrawal of pseudopodia. Subsequently the zone of outgrowth of the culture splits up into detached elements, comprising one or more cells. This whole process is not lethal to the cells and is, to a great extent, reversible.

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Production of Polycythemia in Rabbits by Anoxia and Cobalt.*

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(Introduced by D. R. Drury.)

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A need for polycythemic rabbits as experimental animals led to the consideration of methods of producing the state of polycythemia in this species. There are at least 2 experimental methods for inducing this condition. The older and better known of these is the response to anoxia induced by high altitude; another is the administration of cobalt salts.

Although the stimulating effect of lowered oxygen tension is well known and has been extensively studied, its mechanism is not as yet clearly understood. The effect has been noted in many animals other than man.[1] These include monkeys,[2] dogs,[3] rats[3] and rabbits.[3-5]

Armstrong and Heim[4,5] reported on the exposure of young male rabbits for 4 hours daily 5 days a week to pressures equivalent to 18,000 feet. In addition to this treatment the animals were subjected to an altitude tolerance test on the 7th day. This tolerance test involved rapid decompression to the point where the animals became unconscious. The equivalent altitude attained was noted and the animal immediately returned to ground level. In the 3rd week of their experiment a deterioration set in and the hemoglobin and hematocrit levels depreciated to lower than the original values. They concluded that rabbits were quite unsatisfactory for this work.

Later Thorn, Jones, Lewis, Mitchell and Koepf[3] conducted a series of experiments in which they exposed rabbits to pressures equivalent to 18,000 and 25,000 feet for 4 hours a day 7 days a week. The 18,000-foot animals were continued for 5 weeks whereas the 25,000-foot experiment was discontinued at the end of 3 weeks because of excessive fatalities. Those exposed to 18,000 feet showed a temporary delay in growth, together with a slight drop in the concentration of serum, sodium chloride and CO_2 combining power and a decrease in the weight of the thymus gland. These changes were accompanied by an increase in O_2 capacity of the blood, in the hematocrit values and in the adrenal gland weight. Seventy-five percent of those exposed to 25,000 feet died of hemorrhage into the lungs and/or herniation of distended loops of the intestines into the thoracic cavity. There were no fatalities in the 18,000-foot group, nor was there evi-

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4 Armstrong, H. G., and Heim, J. W., *J. Aviation Med.*, 1938, **9**, 45.

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dence of degeneration in the hemopoietic system.

It may be noted that little is known of the mechanism or the optimum conditions for producing polycythemia by this method.

Another technic for producing polycythemia, whose mechanism is however even less clearly understood, was reported by Waltner and Waltner.[6] They induced polycythemia by the administration of cobalt. This method has been used on several species but was found to be slow and sometimes accompanied by toxic symptoms. Frost *et al.*[7] produced a temporary polycythemia in adult dogs by including cobalt in the food. However, they found this toxic to young growing dogs. On the other hand Stanley, Hopps and Hellbaum[8] reported the development of polycythemia in rats by a subcutaneous injection without unfavorable symptoms.

Methods. It was decided to study the possibilities of both the above mentioned technics for producing experimental polycythemia. The routine of the cobalt injections was patterned after that employed by Stanley *et al.*[8] The exposure time at altitude was materially increased above the time used by previous workers, but remained intermittent. This allowed the animals some time for recuperation under more nearly optimum living conditions at sea level. Altitude and cobalt stimuli were tried separately and together on immature male rabbits. Twelve animals, averaging 3½ lb. were divided into 4 groups of 3 rabbits each. Group I served as controls remaining at sea level pressures and without cobalt injections. Group II was subjected to an ambient pressure equivalent to an altitude of 20,000 feet, 16 hours a day, 5 days a week. Group III was injected subcutaneously with 1 ml of sterile cobalt solution per pay 6 days a week, but remained at ground level (300 feet above sea level). Group IV was injected subcutaneously with 1 ml of cobalt solution per day 6 days a week and was subjected to a pressure equivalent to 20,000-foot altitude 16 hours a day 5 days a week. The cobalt solution consisted of 10 mg $CoCl_2{:}6H_2O$ per cc made up to isotonicity with NaCl. This was injected subcutaneously into a shaved area of the back and shoulders. The altitude was simulated in a decompression chamber. The altitude animals rested at ground levels (300 feet) each day for 8 hours and over Saturday and Sunday night, a period of 56 hours. The altitude "flights" were continued for 10 weeks. This was followed by a 2½-week ground level observation period.

The animals were weighed and sampled twice a week, on Mondays at the end of the 56-hour sea level resting period and on Thursdays after 3 of the 5 successive night flights of 16 hours. The blood samples were taken from the marginal ear vein. Hemoglobin was determined by the acid hematin method with a Klett Summerson colorimeter. Hematocrit values were measured in Wintrobe tubes. Plasma volume was determined by the dye method using T-1824.[9] The plasma volume was measured only 4 times during the course of the entire experiment. These were, first during the prestimulus period, next after 3 weeks of exposure to altitude and/or cobalt, then after 10 weeks of stimuli and lastly at the end of the poststimuli observation period. The dye determination technic consisted of the injection of a weighed amount of a 1% dye solution in isotonic saline. After 8 minutes a blood sample was taken, using 1.6% sodium oxalate solution as an anticoagulant. The blood sample-oxalate ratio was determined in each case by weighing and the ratio used to correct the readings obtained. The intensity of the blue color in the plasma was measured on a Beckman quartz spectrophotometer, using the 620 mμ absorption band.

Results. The groups of animals employed were too small to permit satisfactory statistical analysis. However, the response to the

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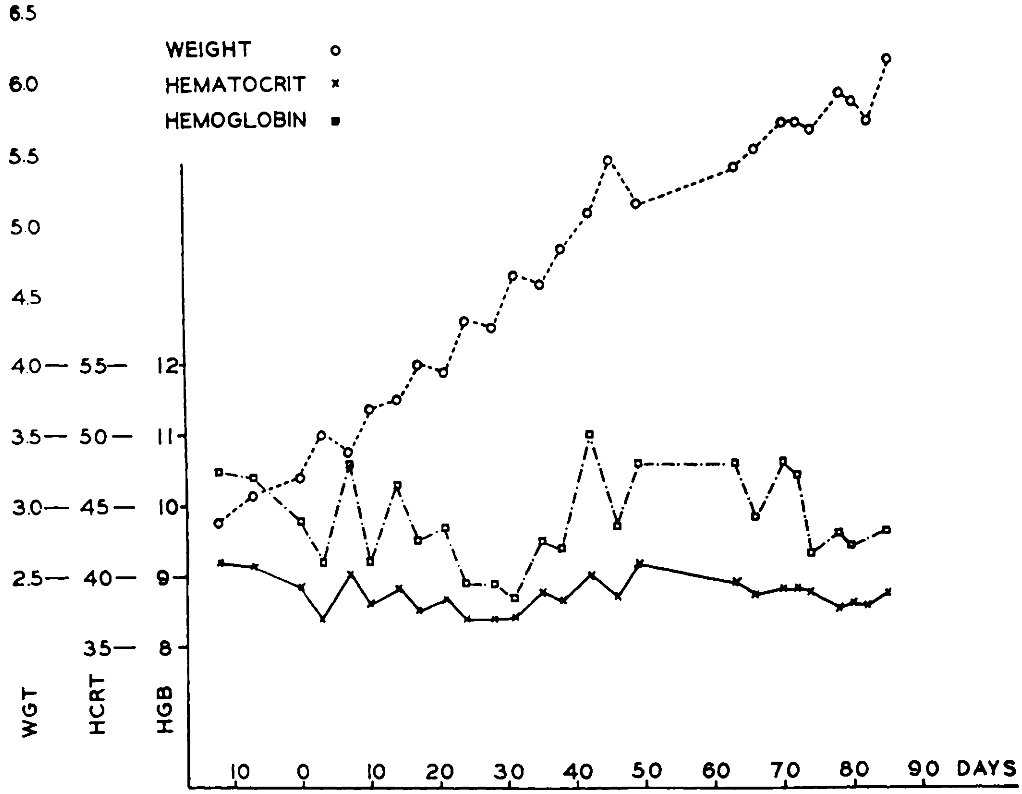

FIGURE I SEA LEVEL CONTROL

Weight, hematocrit, and hemoglobin averages of the control rabbits.

stimuli was definite and conclusively demonstrates the possibility of producing polycythemia in this species without deterioration.

Fig. 1 shows the average of the weight, hemoglobin and hematocrit values of the control group. (Only 2 animals are reported in this group. One was stolen on the second week of the experiment.)

The response to altitude alone is shown in Fig. 2. Each parameter represents the average value for 3 animals. (No fatalities). The exposure to altitude prompted a decrease in weight, followed by a period of slow recovery with a final resumption of a growth rate equivalent to that of the controls. Hemoglobin and hematocrit values both showed a rapid increase during the weekend resting period after the first exposures to altitude. This sloped off; first into a period of little change and then into a gradual climb which persisted as long as the stimulus was applied. The decrease in hemoglobin and hematocrit from 25th to 35th day coincided with a similar depression in the control group and a period of high summer heat. It is to be noted that, during the control period following the altitude exposure the hemoglobin and hematocrit values returned rapidly toward the normal levels.

One of the cobalt-injected, ground level group, died apparently from the toxic effects of the cobalt combined with severe anorexia. The data in Fig. 3 represent the average of the values obtained from the 2 remaining animals. The weight curve generally follows that of the controls. The hemoglobin value dropped slightly when the injection started, followed by a gradual increase for the period of the injections, but did not attain values comparable to those in the animals subjected to altitude. The return to normal after in-

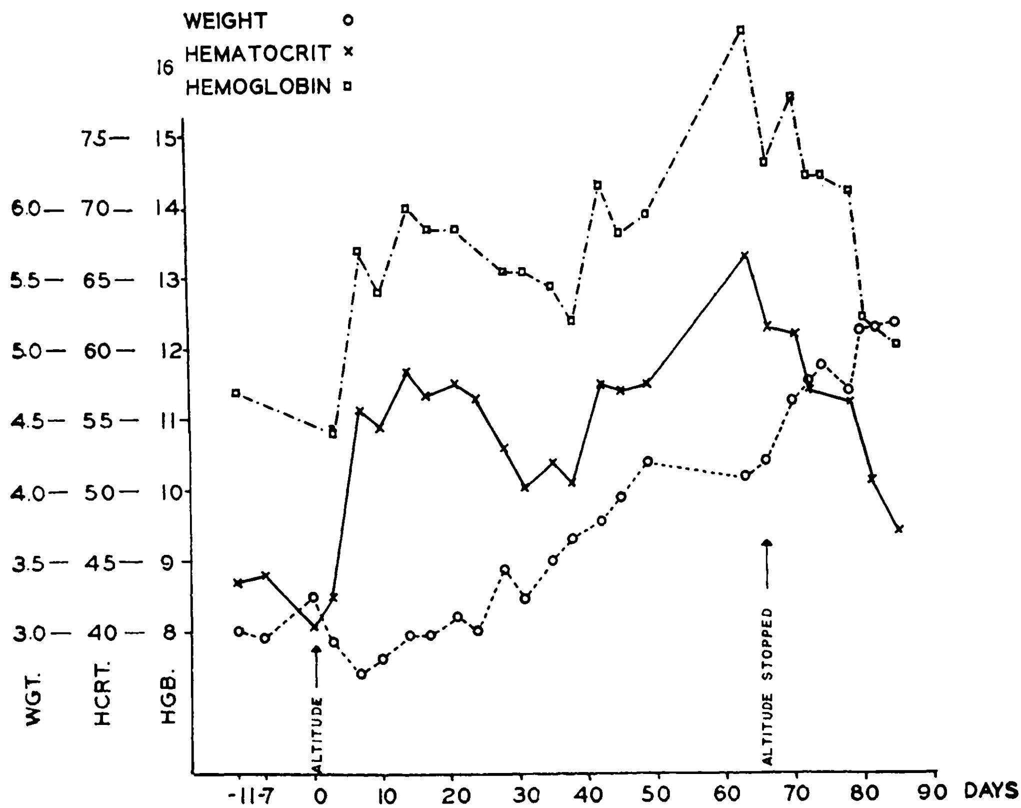

FIGURE 2 EFFECT OF ALTITUDE
Weight, hematocrit, and hemoglobin averages of rabbits having had ten weeks of intermittent altitude exposure.

jections were stopped, was slower than that found with the altitude rabbits.

Two of the 3 animals receiving combined altitude and cobalt treatment died: one apparently from the toxic effects of the cobalt, the other from the combination of toxic effects and peritonitis. Sterile abscesses developed on all 6 animals receiving cobalt injections at or near the site of the injection. The larger of these were opened and drained; however, no infections developed in these areas. The curves for the one surviving altitude and cobalt animal are shown in Fig. 4. They closely resemble those found in the Group II or altitude only animals, with the exception of the absence of the depression of hemoglobin and hematocrit values from 25th to 35th day. This may be due to individual variation. The differences are not sufficient to indicate that the 2 stimuli are additive in producing polycythemia. However, a more prolonged experiment might show this to be true.

The plasma volume and whole blood volume data are given in Table I, as the average of each group in per cent increase above the prestimulus period. In this manner each animal is compared to himself at the start of the experiment, and as all the animals were still growing all values are positive. The significance can then be shown by comparing the various groups to the control group. The whole blood volume values were obtained from the plasma volume and the corresponding hematocrit value.

The values in parentheses show the per cent change found during each period (*i.e.* the increase or decrease in percentage of each period as compared with that of the preceding period). From this one can more easily

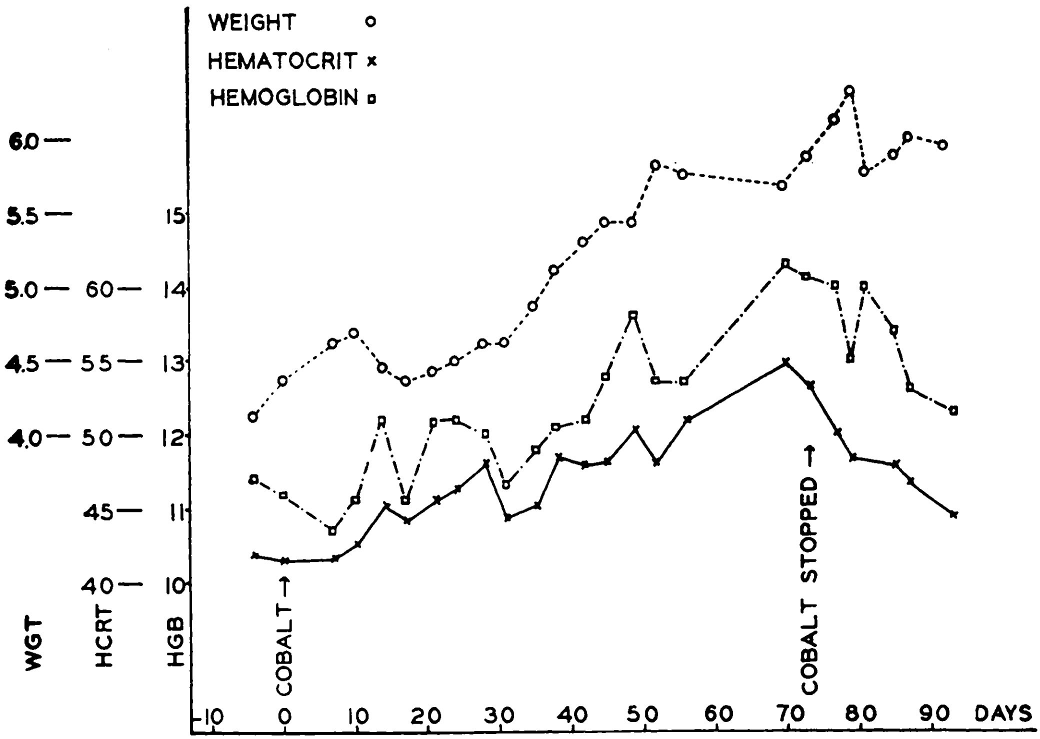

FIGURE 3 EFFECT OF COBALT
Weight, hematocrit, and hemoglobin averages of rabbits given daily cobalt injections.

follow the progressive changes at each period of the experiment.

After 3 weeks of stimuli (Table I, Column 4) there was no significant increase in whole blood volume above that of the controls. However, the hematocrits of those exposed to altitude showed an increased 45% in red cell volume at the expense of the normal increase in plasma volume. The animals exposed to altitude showed almost no change in plasma volume (3% as compared to 50% for the controls, Table I, Column 1).

During the ensuing 7 weeks of exposure to stimuli, the increase of plasma volume in the experimental animals was comparable to the increase in the controls (from 22.5% increase to 32.1% increase). At the same time the increase in the whole blood volume of the altitude animals (66% and 68%) was more than double the increase of the controls (24.2%). This suggests that the development of polycythemia may have 2 phases or processes; an initial immediate and rapid one involving an increase in the cellular phase at the expense of the plasma volume and a second more gradual step during which the plasma volume increases at a normal rate together with a further increase in the red cell volume.

The changes occurring after removal of the stimuli are for the most part in the red cell component of the blood. The plasma volume of the altitude animals increased above that of the controls and there is a large drop in the total blood volume. The behavior of the altitude plus cobalt animals was similar to that of the controls as far as the plasma volume changes were concerned but there was a large drop in whole blood volume.

Even after 10 weeks of cobalt injections Group III did not have a greater whole blood volume than that of the controls. However, they showed only a moderate polycythemia which was attained at the expense of the plasma volume. Yet after the stimulus had

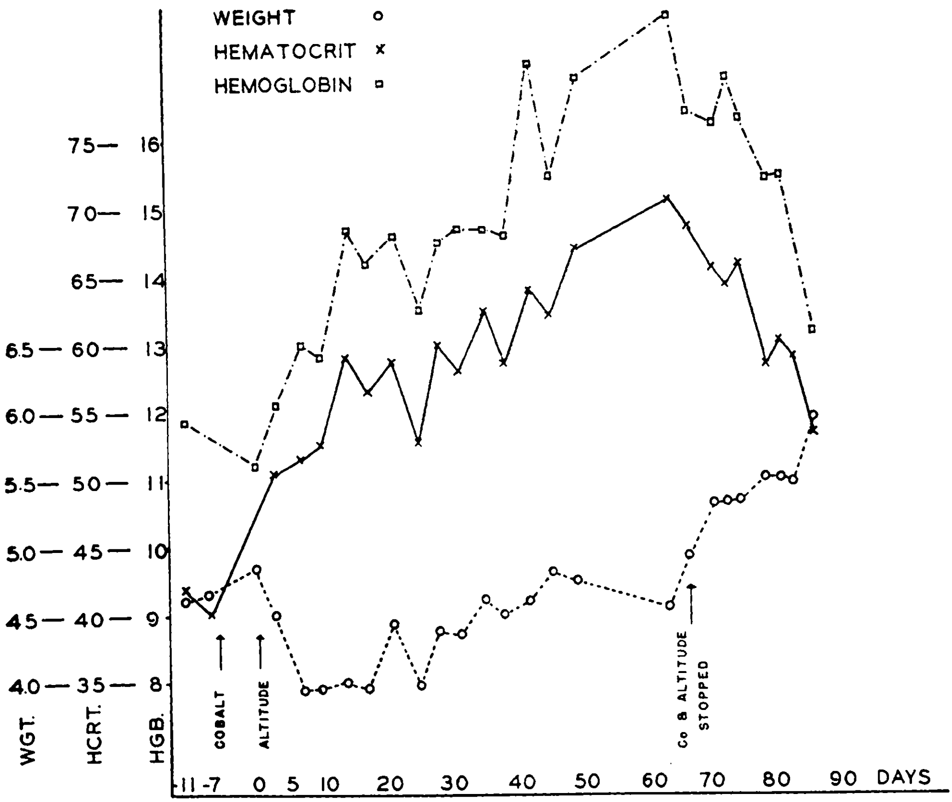

FIGURE 4 EFFECT OF COBALT AND ALTITUDE

Weight, hematocrit, and hemoglobin values of rabbit surviving ten weeks of intermittent altitude and cobalt.

TABLE I.
Percent Increase Above the Pre-experimental Value.

	Plasma volume			Whole blood volume		
	After 3 weeks of stimulus	10 wks of stimulus	2½ wks of recovery	After 3 wks of stimulus	10 wks of stimulus	2½ wks of recovery
Column No.	1	2	3	4	5	6
Control	+50.0%	+72.5%	+86.0%	+39.6%	+ 63.8%	+74.5%
		(+22.5)*	(+13.5)		(+24.2)	(+10.7)
Cobalt and altitude	+ 3.3	+28.0	+33.0	+40.4	+107.0	+60.0
		(+24.7)	(+ 5.0)		(+66.6)	(—47.0)
Altitude	+ 2.6	+34.7	+57.7	+33.7	+102.2	+69.3
		(+32.1)	(+23.0)		(+68.5)	(—32.9)
Cobalt	+12.3	+40.5	+33.5	+26.0	+ 63.3	+40.0
		(+28.2)	(— 7.0)		(+37.3)	(—23.3)

* Values in parentheses show the changes in percent increase above the values of the previous period.

been stopped, there was a decrease in plasma volume as well as total red cell volume.

This either indicates that the development of cobalt and altitude polycythemia involves different mechanisms of response, or that the cobalt animals at 10 weeks had only developed to a stage comparable to the first 3 weeks of altitude exposure.

Conclusion. Polycythemia can be rapidly induced in rabbits by exposure to altitudes of 20,000 feet 16 hours a day, 5 days a week. It would appear that there is no additive 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

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