the letter "N" indicates the neutral point as determined by this method. A wide zone of precipitation occurred when the antitoxic serum prepared against the 20-day formolized filtrate was mixed with either its homologous toxic filtrate or with the 20-day filtrate of the atoxic variant. The antiserum prepared against the 20-day filtrate of the atoxic variant also produced marked precipitation in the presence of both the homologous filtrate and of the 20-day filtrate of the toxic strain. On the other hand, in the case of the antitoxin obtained against the 4-day filtrate of the toxic variant no precipitation occurred in the presence of the various filtrates tested, although this serum neutralized the toxin in the *in vivo* tests. Similarly, no precipitation occurred when the antiserum prepared against the 4-day filtrate of the atoxic variant was combined with the respective antigens.

The results of these experiments indicate that (1) the filtrates of the old cultures of the toxic variant contain bacterial protein in addition to the toxin, thereby stimulating the production of both antibacterial antibodies and antitoxin; (2) that the filtrates of the young cultures of the toxic variant are relatively free from bacterial protein and hence their antisera contain antitoxin and no detectable antibacterial antibody; (3) that precipitation with tetanus-toxin and antitoxin does not result primarily from the union of toxin and antitoxin.

Similar experiments with staphylococcal toxin and antitoxin will be reported subsequently.

8699 C

Effect of Dinitrophenol on Agglutinin and Complement Titer.

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The prompt and striking pharmacologic actions, sustained increases in metabolism and body-temperature, produced in man and animals by dinitrophenol, suggested its employment to test the influence of these altered functions on immunologic reactions. Rabbits 1, 2, 3 and 4 were given daily or twice daily, subcutaneous injections of 10 mg. gradually increasing to 24 mg. of dinitrophenol (2.4) mg./k. body-weight over a period of 37 days. The solution was made up in 3% volume with ½ weight of $NaHCO_3$. On the

first, fourth, eighth and eleventh days of the dinitrophenol administration the rabbits and 2 control rabbits, 5 and 6, each received a subcutaneous injection of one cc. of a heat-killed suspension of *B. paratyphosus* B. for the production of agglutinins. The animals gained weight and evidenced no apparent effect of the drug other than a rise of 2 to 3 degrees in temperature. Quantitative agglutination-tests failed to show any influence of the dinitrophenol on the rate or extent of production of agglutinins (Table I).

TABLE I.
Agglutinin Titer.

	Test Rabbits (DNP)					Control Rabbits (No DNP)		
Days	1	2	3	4	Av.	5	6	Av.
0	0	0	0	0	0	0	0	0
7	600	640	640	1280	800	2560	640	1600
14	1600	1600	1600	1600	1600	3200	3200	3200
16	800	800	1600	6400	2400	3200	3200	3200
18	1600	1600	6400	6400	4000	3200	—	3200
22	1600	—	3200	1600	2100	—	3200	3200
28	800	—	3200	800	1600	1600	1600	1600
37	800	800	—	800	800	—	400	400

At the end of the administration-period the animals were given a single larger dose of dinitrophenol. Rabbit 1 received 35 mg./k., the temperature rose to 105.6°F. and no change in agglutinin-titer occurred. Rabbit 2 was given 35 mg. per kilo, the temperature increased to 106.3°F. with no change in titer. Rabbit 3 survived the injection of 40 mg./k. with a rise in temperature of 4.1°F. and no significant alteration in titer. Rabbit 4, given 35 mg./k., responded with an increase in temperature from 102.2 to 105.4°F., with no change in titer.

Five of the animals were allowed to rest until the agglutinin-titer declined, from 4 to 11 months, and were then given another large dose of dinitrophenol to see whether a secondary advance in titer would result. Temperatures of from 105° to 110°F. were attained. The agglutinin-titer was unchanged except in one rabbit in which it increased from 1:600 to 1:2000.

The effect of dinitrophenol on the complement-content of rabbits 1, 2 and 6 was also determined. The complement-titer of rabbit 1 was determined before and after the administration of 30 mg./k. of dinitrophenol, followed by 14 mg. per kilo, when the temperature had reached 106°F. Table II shows a very slight decrease in complement at 107.6°F. The test was repeated, using the native hemolytic amboceptor present in the rabbit-serum. Here

TABLE II.
(Rabbit 1.)

	Undiluted unheated serum											Undiluted inactivated serum				
	0.1 cc. amboceptor added				No amboceptor added							No amboceptor added 0.1 cc. (1-4) guinea pig serum				
Temp.	0.05	0.1	0.2	0.3	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.01	0.02	0.03	0.04	0.10
102.5	—	++	++++	++++	—	—	—	+	++++	++++	++++	+	++	++	+++	++++
106.0	—	++	++++	++++	—	—	—	—	+	+++	++++	+	++	++	+++	++++
107.6	—	+	++++	++++	—	—	—	—	—	+	++++	+	++	++	+++	++++
108.4	—	+	++++	++++	—	—	—	—	—	+	++++	+	++	++	+++	++++

the difference was more marked. To ascertain whether the change was in the complement or native hemolysin the serum was inactivated and guinea pig's complement added. Here, the uniform titers indicate that the change observed after the injections of dinitrophenol was in the complement (Table II).

Rabbits 2 and 6, following the injection of 30 mg./k., exhibited no essential change in complement titer.

Tests of uninjected rabbit's serum revealed no significant diurnal variation in complement or hemolysin.

Summary. Subcutaneous administration of dinitrophenol in varying amounts from 10 mg. to 24 mg./k., over a period of 37 days, had no apparent effect on the production of agglutinins for *B. paratyphosus* B in rabbits. Large amounts, 30 to 40 mg. per kilo, sufficient to increase the temperature several degrees, did not significantly alter the agglutinin-titers. Similar large doses given after a rest period of from 4 to 11 months failed to cause a secondary rise in titer except in one instance. A very slight decrease in complement-content took place in 2 of 3 experiments following large doses of dinitrophenol. The above results did not encourage more extensive investigation.

8700 P

Vasomotor Response of Non-Hypertensive Individuals to a Standard Cold Stimulus.

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Hines and Brown[1] have described a standard test for the study of vasomotor reactions based on the responses of the blood pressure to a cold stimulus. They are of the opinion that "excessive" responses to this test indicate a hypersensitive sympathetic nervous system. Assuming that the principal abnormality in essential hypertension is a hypersensitive sympathetic nervous mechanism, they have considered non-hypertensive individuals with "excessive" responses potential candidates for hypertension. They suggest that essential hypertension develops only in individuals manifesting "ex-

[1] Hines, E. A., Jr., and Brown, G. E., *Ann. Int. Med.*, 1933, **7**, 209.