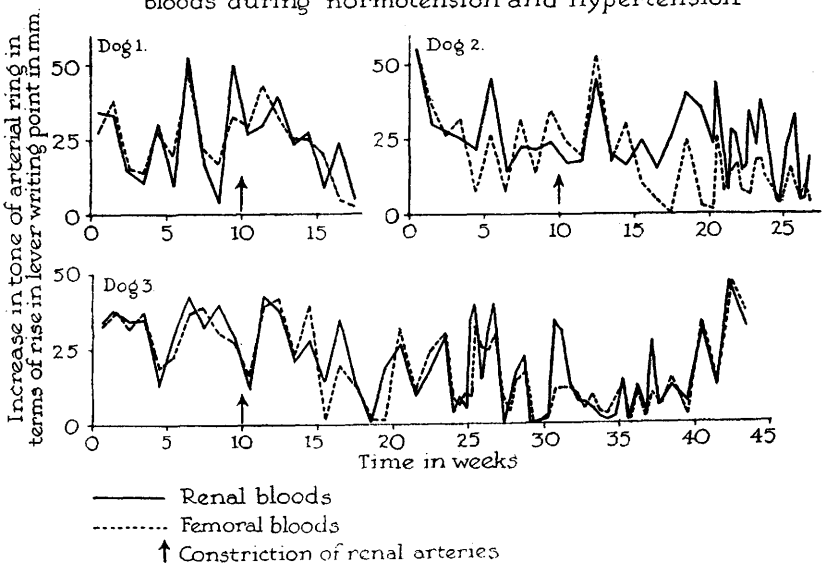
Fig. I

Tone-augmenting effects of renal vein and femoral artery bloods during normotension and hypertension



method, as previously outlined[1] are such that positive results only would be conclusive.

Conclusions. 1. The vasoconstricting effects of heparinized renal vein and femoral artery bloods from three dogs before and after the production of renal (Goldblatt) hypertension were studied on arterial segments from the mesenteric arteries of beeves. 2. No significant differences were found either during normotension or hypertension. 3. These results do not support the presence of a pressor substance in the renal vein and systemic bloods of Goldblatt dogs but by no means rule out the probability.

13451 P

Changes in Antitryptic Power of the Blood Associated with Anaphylaxis in Guinea Pigs.

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That the natural antitrypsin of body fluids plays a determining

rôle in anaphylactic reactions was postulated many years ago,[1, 2, 3] but the theory has since been almost totally neglected, probably because of the lack of a simple reliable quantitative test for antitrypsin. The present paper records preliminary results of a study in which changes in serum antitrypsin associated with guinea pig anaphylaxis were determined by a new procedure developed by the writer.[4]

Methods and Materials. From a group of 30 male guinea pigs sensitized to egg white blood samples were taken aseptically by heart puncture, without anesthesia, during the incubation period, just before and just after introduction of the shocking dose of the antigen, at death, and, in the case of surviving animals, at various intervals of time after the antigen injections. Normal guinea pigs, inoculated intracardially with egg white as controls, were similarly bled. Blood specimens before and immediately after intracardial antigen injections were obtained in the following way: (1) heart was punctured and the first sample was drawn into the syringe, which was then detached, leaving the needle in the heart, (2) a second syringe containing egg white was connected to the needle and the shocking dose was injected, (3) this syringe was now removed and replaced by a third syringe into which was drawn at once the second sample of blood. Sera collected from the clotted blood specimens were centrifuged until perfectly clear and stored in the refrigerator in sealed sterile tubes until tested.

The antitryptic power of these sera was determined by the film-disc method, following a procedure much simplified and improved over that originally described.[5] Photographic film which has been exposed, developed and hardened in a particular manner is cut into discs 5 mm in diameter. These discs are floated gelatin-side down upon graded dilutions of a standard, stable trypsin solution[6] and upon mixtures of the same trypsin solution in greater concentrations with the serum under test. After incubation at 40°C for an accurately timed period (about 10 minutes) the reactions are stopped, and the dried discs are finally mounted upon a filing card which thus serves as a permanent record. The titer of tryptic activity is expressed in terms of milligrams of trypsin per cc in the dilution which is just strong enough to cause complete clearing of the film (*i. e.*,

[1] Bronfenbrenner, J., PROC. SOC. EXP. BIOL. AND MED., 1915-16, **13**, 42.

[2] Bronfenbrenner, J., and Schlesinger, M. J., *J. Immunol.*, 1918, **3**, 321.

[3] Rusznyak, S., *Deutsch. med. Wchnschr.*, 1912, **38**, 168.

[4] A detailed description of the method to be presented elsewhere.

[5] Burdon, K. L., and Lafferty, C., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 787.

[6] Burdon, K. L., *Science*, 1941, **93**, 91.

TABLE I.

Blood specimens	No. of G.P.	No. of Spec.	Antitryptic index			
			Lower	Higher	Unchanged	Avg
Normal guinea pigs (never inoculated)	8	8				13.6
Normal guinea pigs, 10 sec. to 3 min. after i. c. inoculation of egg white (no reaction)	8	8	1	1	6	13.5
7 days after last of 2 i. p. (sensitizing) inj. of egg white	14	14		V		31.4
14-21 days after last sensitizing inj. and just before inoculation shock dose of egg white	13	13	V			29.6
From sensitized guinea pigs, 10 sec. to 2 min. (avg < 1 min.) after inoculation of shock dose of egg white	15	18	11	2	5	24.6
Just after death from anaphylactic shock (death in 3-5 min.)	14	14	2	3	9	27.5
16 hrs to 5 days after sublethal desensitizing dose of egg white	9	26	2	15	9	35.0

complete digestion of the gelatin layer), and the difference in the titer in the presence and in the absence of a particular serum represents the antitryptic power of that serum. The final figure used to express the *antitryptic index* of a serum sample is obtained by multiplying this difference in titer by the dilution of the serum. For guinea pig sera the dilution factor is 20, since all specimens are diluted 1:20 for the test.

Results. The findings in tests of 101 serum specimens are summarized in Table I.

It will be seen (1) that the antitryptic titer increased markedly as a consequence of the sensitizing doses of egg white, so that at the end of the incubation period the average index was about twice that of the normal, unsensitized animals (a finding not reported previously); (2) that the majority of serum specimens obtained within a minute after intracardial injection of the antigen into *sensitized* guinea pigs showed a decided drop in antitrypsin, while typical symptoms of shock were developing, in contrast to normal animals similarly injected which showed no symptoms and no fall in serum antitrypsin; and (3) that the serum just after death gave an average antitryptic index only slightly higher than the same animals showed before the antigen injection, but surviving guinea pigs had in their sera an especially high content of antitrypsin during the following few days. Several of the latter were shown to be antianaphylactic.

While these data are insufficient to permit final conclusions they demonstrate that definite changes in serum antitrypsin accompany

anaphylactic reactions. They indicate that a sudden drop in antiferment is associated with the initiation of shock, and that a significant increase in antitryptic elements occurs during reactions, so that the serum of surviving, antianaphylactic animals possesses an unusually high antitryptic power.

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Evaluation of Bactericides by Egg Injection Method with Special Reference to Development of Technic.*

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Tissue culture methods[1] have been used to measure the toxic action of bactericides through their influence on groups of cells *in vitro*. Such technics, however, do not properly involve the complexity of organ and tissue interrelation which exists in the intact animal, adult or embryonic. The avian embryo was chosen principally for these experiments because manipulation with the whole animal is afforded *in vivo* and because large amounts of albuminous material with which the bactericidal agent might come in contact are present.

General Method. Immediately preceding injection, fertilized eggs of known origin† were candled to assure vitality. The area on the shell opposite the embryo was treated with tincture of iodine and a small hole was drilled‡ through the marked area to expose the *membranae putaminis* (the membrane lining the egg shell). Throughout the manipulation the egg was kept on its side with the marked area uppermost. Using sterile technic, dilutions of the antiseptic made with sterile distilled water were rapidly injected into the egg through a ¼ inch, 27 gauge needle. The hole was then sealed with a drop of sterile paraffin at solidifying temperature.

The eggs were incubated at 38.5°C in an oblique position, with

* Presented before the Pharmacy Subsection of the Medical Division of the American Association for the Advancement of Science, Philadelphia, Pennsylvania, December 28, 1940.

1 Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935 and 1936, **32**, 265, 937, 1057, 1119, 1481; **33**, 8, 393; **34**, 371.

† Blood-tested White Leghorns.

‡ Dremel "Moto-Tool" Model No. 1, using burrs No. 107. Manufactured by the Dremel Tool Company, Racine, Wisconsin.