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Studies on Auto-Digestion. IV. Antitrypsin and Clotting Time.

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In a previous paper (Necheles, Ling and Fernando) we stated, that the trypsin inhibiting factor of the serum is not an antibody and that the survival of living tissue exposed to the action of trypsin¹ does not depend upon immunization against the ferment, but on some unknown factor.

In the course of this previous work we were struck by the fact that delayed clotting of the blood was frequently associated with a high "antitryptic" titer. We therefore tested in a series of experiments the possible relationship between clotting time and "antitryptic" titer.* The clotting time and "antitryptic" titer of the blood of 2 normal dogs (No. 870, 9.7 kg., and No. 871, 9 kg.) was determined. The blood was withdrawn daily (in the morning) by heart puncture from the left ventricle (a paraffined syringe and needle was used†). The dogs were fed in the afternoon; the food consisted mainly of carbohydrates. In the course of a week, the clotting time varied from 7' 30" to 13' and the "antitryptic" titer from 0.2-0.4 in dog 870, and from 7' 15" to 11' 30" and 0.2-0.4 in dog 871, but no relationship could be found between these 2 values. There was no difference between the "antitryptic" titer of blood which had clotted in plain and that in paraffined glass tubes, although there were differences of 30 to 100% in clotting time. Blood

* The same methods as previously described were used.^{3, 5}

¹ Necheles, H., Ling, J., and Fernando, F., *Am. J. Physiol.*, 1926, lxxix, 1.

† Occasionally venous blood was drawn, which clotted much quicker; its "antitryptic" titer was not different from the average titer of arterial blood.

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in paraffined glass tubes to which tissue fibrinogen was added clotted about 10 times quicker than the control but had the same "antitryptic" titer.

In order to see whether increasing metabolism would influence clotting time and titer, both dogs were treated with subcutaneous injections of thyroxin, till pulse rate and body temperature indicated full action of this drug. In dog 870 there were insignificant variations of the titer between 0.3 and 0.5 and no change of clotting time. In dog 871 the titer went up from 0.3 to 20.0‡ without change in clotting time. This experiment confirms our former statement about the inconsistency of changes of the "antitryptic" titer.²

Since running produces decrease of clotting time, 6 experiments with exercise were carried out on 4 dogs. After 10 minutes running there was in all cases a marked hastening of clotting; in one case there was a decrease of the titer (from 0.3 to 0.1) in another case an increase (from 0.3 to 0.6), in the other experiments the changes were insignificant.

In one apparently normal (starving) dog we found free trypsin in the serum. Unfortunately the animal was killed the next day, but the autopsy did not show anything peculiar; this is confirmatory of statements made by other authors.^{3, 4} Meat meals were found to decrease the titer down to zero in dogs; a fat meal brought it down from 0.5 to 0.1, while carbohydrate (rice) was without influence. The changes in the clotting times after the different meals were in no apparent relationships to the varying titers.

There is no relationship between "antitryptic" power of the serum and clotting time of the blood. The inconsistency of changes of the "antitryptic" titer is confirmed.

‡ The "antitryptic" power of serum with a titer higher than about 1.0 is obtained by multiplying the titer with the degree of serum dilution. Since this procedure assumes a linear relationship between serum concentration and titer, we determined the titer in various dilutions of the sera of 3 different normal dogs. It was found that somewhat above 2% serum concentration the titer did not increase but remained constant; this was apparently due to absorption of the trypsin by the serum proteins. Below a certain dilution of the sera (1.35-0.8%) there was no more "antitryptic" action; this is apparently the lower limit of exactness of the test. Between 0.8-2% the relationship between serum concentration and "antitryptic" power was a direct one. Our calculation of the titer (here and in previous articles²) in sera with a very high "antitryptic" power is thus only an approximate one.

² Necheles, H., and Fernando, F., *Am. J. Physiol.*, 1926, lxxix, 9.

³ Joemann, G., and Kantorowicz, A., *Z. f. klin. Med.*, 1908, lxvi, 153.

⁴ Müller, E., and Joemann, G., *Munch. med. Wchnschr.*, 1906, liii, 2002.