



THE ACTION OF BLOOD SERUM OF THE DOG IN A  
CONDITION OF TETANIA PARATHYREOPRIVA ON  
VOLUNTARY MUSCULAR TISSUE COMPARED WITH  
THAT OF NORMAL SERUM. By B. A. HOUSSAY, Buenos Aires.

(Received for publication 25th October 1917.)

IN a recent paper G. M. Wishart<sup>1</sup> gives the results of an attempt to obtain a qualitative estimation of the guanidin in blood by immersing frog muscles in serum. He found that, of six cases observed, the serum of three dogs which had been subjected to parathyroidectomy produced tremors in the muscles of the frog: whereas in three other cases there were no tremors. In some there was prolongation of the normal contraction—an effect similar to that produced by guanidin alone. The serum of a cat affected by tetania parathyreopriva also caused tremors in frog muscle: the serum of normal animals (six dogs and four cats) always proved inactive. The conclusion he draws is that “in certain cases the serum of parathyroidectomised dogs and cats acts upon the muscles of the frog in the same way as do dilute solutions of guanidin and methylguanidin. But the variations in the susceptibility of the muscles of different frogs renders this biological test unreliable.”

We have undertaken the study of the behaviour of the muscles of our common South American laboratory frogs and toads in presence of blood serum of dogs both normal and in the condition of tetania. We were led to do this from the fact that our common frog, *Leptodactylus ocellatus* (L.), differs considerably from European frogs in the behaviour of its muscular tissue to drugs, e.g. to curari. The muscles of our common toad, *Bufo marinus*, are, on the other hand, more like those of European laboratory frogs in their behaviour to drugs.

Our experiments were made either with defibrinated blood or (more commonly) with serum; the results with both fluids having been always found to be concordant. Eight normal dogs and seven parathyroidectomised dogs were employed. In six of the seven parathyroidectomised dogs a sample of blood was drawn just previous to the operation for thyro-parathyroidectomy in order to ascertain the action of the normal serum of the particular animal upon muscle. All the operations were performed under chloroform anæsthesia, with the exception of one phlebotomy—which was

<sup>1</sup> Quart. Journ. Exper. Physiol., 1917, x, 355.

made without anæsthesia in a case of severe tetania (dog)—and two in which the anæsthetic used was ether.

Fragments of muscles of the dog taken from the legs or diaphragm never showed tremor, whether immersed in normal serum or in the serum obtained from dogs in a condition of tetania. The gastrocnemius, sartorius, and other muscles, employed either entire or cut into strips, obtained from the thigh of *Leptodactylus ocellatus*, also never exhibited any tremor. On the other hand, the muscles of the toad (*Bufo marinus*) tended to exhibit tremors, as subsequently detailed, but the effects varied greatly in different subjects. The reactions which they showed allow them to be classified as follows:—

1. Animals whose muscles are insensitive to any kind of serum, even that in which the muscles of other toads react strongly. Such animals are frequent.

2. Animals the muscles of which show spontaneous tremors when they are immersed in pure Ringer or in the serum of dogs, either normal or in the condition of tetania. These also are frequent.

3. Animals the muscles of which exhibit tremors only in the serum of dogs with severe tetania. This condition is quite exceptional, only two of our toads having shown it.

4. Muscles in which, besides tremors, contracture was produced. In the second and third groups above enumerated it sometimes happened that a certain amount of contracture was to be seen in the muscles in which tremors were produced.

Method.—The method employed to investigate the reaction was the immersion of either the whole muscle or of strips (a) in the serum of a normal dog, and (b) in the serum of a dog afflicted with tetania parathyreopriva. When such immersed muscle is observed it is noticeable that sometimes only small fibrillations are seen; this represents a minimal amount of tremor. But if the serum is active, local tremors make their appearance; these may produce curvation of the fragments of muscle, which even move about like small wriggling worms. Such tremors generally maintain a certain degree of rhythm; they increase in intensity for a few minutes, but usually begin to diminish after fifteen minutes; at the end of thirty minutes they have generally entirely stopped. They appear to be strongest between the temperatures of 10° and 15° C., decreasing as the temperature is lowered (to 0·5° C.) and also as it is raised (to 35°–37° C.). A sudden change of temperature is usually accompanied by an increase of the tremors.

The following gives details of some of the experiments:—

- I. Dog 1, 11,200 grm.—Sixteen days after operation. Has not developed tetania. Is bled under chloroform anæsthesia at the same time as dogs Nos. 3 and 5, and the samples of serum are compared with one another in their action on muscle. The serum of this dog has no action on the muscles of the frog, toad, or dog itself.

Dog 3 (7/8/17), 10,000 grm.—Intense tetania three days after operation. Sample of blood taken under chloroform. The normal serum from blood collected during the operation is inactive, but the tetania serum is very active when tried on the muscles of three toads. It has no action on the muscles of the frog.

Dog 5 (7/8/17).—Tetania two days after operation. Bled on the third day, when the animal was in a condition of pronounced tetania. Both the normal serum obtained before the operation and the serum obtained when the animal was in a condition of tetania fail to produce tremors in the muscles of two toads, several frogs, and one dog (cf. dogs Nos. 1 and 3).

II. Dog 2 (25/7/17), 7000 grm.—Tetania five days after operation, becoming stronger on the seventh day. The animal was then bled under chloroform anæsthesia. The normal serum is found to be inactive for the muscles of the toad, whereas the serum obtained during tetania produces tremors in the muscles of three toads, but not in those of two frogs tested. The influence of temperature was studied in this case.

III. Dog 4 (7/8/17).—Intense tetania. Corneal ulcer appearing on the eighth day. Bled under chloroform anæsthesia, normal serum having been collected before the operation. The latter is found to be inactive, whereas the serum obtained in the condition of tetania produces intense tremors in the muscles of three toads. In this case also the influence of temperature was observed. It was further noticed that the serum produced tremors even after it had been boiled.

IV. Dogs 6 and 7.—Normal serum of dogs Nos. 6 and 7, collected at the operation of parathyroidectomy (under the administration of ether), caused tremors in the muscles of the toad, but none in those of the frog. Samples of the serum were kept aseptically in oil for seven days, after which time the dogs from which they had been taken had developed intense tetania. Samples of blood were then again taken under chloroform anæsthesia. Simultaneously with these, other samples were taken from two normal dogs under the same anæsthetic. On that and the following days the six samples of serum (two from the normal dog, two from dogs 6 and 7 before operation, and two from dogs 6 and 7 in the condition of tetania) were investigated. Several frogs were tested; the muscles of all gave negative results. The muscles of toads showed (a) in one case no reaction; (b) in another case reaction only with serum of dog 6 taken in the condition of tetania; (c) in several cases all the serums tested produced tremors, the reaction being especially intense with the samples of serum which were taken from dogs 6 and 7 in tetania. Of these, the serum of dog 6 in tetania was the most active, but the normal serum of the same animal showed greater activity than the serum of dog 7 in tetania and nearly as much as that of dog 6 itself in tetania. Other normal serums also acted nearly as effectively as that of dog 7 in tetania.

## CONCLUSIONS.

1. The muscles of the South American frog, *Leptodactylus ocellatus*, and of the dog itself are not thrown into tremors by the serum of dogs either normal or in a condition of tetania parathyreopriva.

2. The muscles of the South American toad, *Bufo marinus*, cannot usefully be employed as a reagent to determine whether the serum of parathyroidectomised dogs has convulsive properties, since they show great variability in sensitiveness, and are apt to give twitchings even in normal serum.

3. In five out of six dogs samples of blood obtained during tetania parathyreopriva produced tremors in the muscles of the toad. In another parathyroidectomised dog, which, however, did not exhibit tetania, the serum produced no effect.

4. In four cases samples of serum obtained from normal dogs produced tremors in the muscles of the toad which were to all appearance as intense and persistent as those obtained from the action of the serum of dogs in tetania parathyreopriva. On the other hand, samples obtained from four other normal dogs produced no effect.

