BLOOD CALCIUM DISTRIBUTION IN ANAPHYLAXIS IN THE GUINEA PIG.

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The biological importance of calcium has, of course, long been known; but it has only been comparatively recently, through the stimulus afforded by the findings of the close relationship between certain pathological conditions and disturbed calcium metabolism, that the element has been so extensively studied. The effect of calcium on tissue permeability and irritability, and the rôle attributed to it in blood coagulation is of special interest in the study of anaphylaxis; and the work to be reported here is a study of the variations in the total calcium and in the diffusible and non-diffusible fractions which occur in the anaphylactic guinea pig.

While many determinations have been made of total serum calcium, usually with findings of a constant or only slightly diminished value (1–3), a review of the literature yields no report on diffusible calcium in anaphylactic shock. Blum, Delaville and Caulaert (4) found the diffusible calcium in peptone shock in one dog increased from 5.7 to 7.5 mg. per 100 cc.; and in the rabbit, in which the manifestations of shock were weak, two experiments gave nearly double the values for the diffusible fraction over that found in the control animal. No such study is to be found for the guinea pig, in which, of all the experimental animals, anaphylactic shock is to be demonstrated most uniformly and definitely.

Technic.

For this study 66 guinea pigs were used, including the normal controls. The shocked animals were prepared by direct sensitization with human or horse serum, or passively by the transfer (48 hours) with an anti-horse serum (rabbit). Since the size of the animal precluded preshock bleedings, subsensitized animals, for controls, were injected with horse serum and then killed after varying intervals corresponding to those at which the shocked animals were bled.

In all the groups it was necessary to use the pooled bloods of from 2 to 4 animals, depending upon their size, in order to secure a sufficient sample of serum for analysis. Care was taken, in all cases, that the animals in each group showed the same type of reaction, and that the bleedings were made at the same intervals after injections.

Calcium determinations were made according to Tisdall's modification (5) of Kramer and Tisdall's method (6). The method of Moritz (7) as modified by Updegraff, Greenberg and Clark (8) was used in obtaining the diffusible calcium findings. As a criterion of a proper permeability of the collodion bags, we accepted only those results in which not less than 1 cc. of fluid passed through the membrane and which, of course, gave no tests (biuret) for protein in the diffusate. Nearly all results given in the table are averages of two determinations and all the results were actual ones, *i.e.*, the non-diffusible calcium was not calculated by difference. This we believe to be an added check on the results, for though the possibility has been suggested (8) that some Ca may be lost in the membrane itself, an inspection of the tables will indicate that the sum of the diffusible and non-diffusible calcium is, in general, rather more, instead of less, than the total calcium, indicating that such losses are quite negligible and are more than counterbalanced by the errors inherent in the calcium determination itself.

DISCUSSION.

In the table are listed the results of determinations for total serum calcium, the diffusible and non-diffusible, and the percentage of these of the total calcium. All the figures are placed in one table to facilitate comparison of the normal findings with those for the animals which were subsensitized and did not show shock symptoms and those which showed a marked degree of anaphylaxis.

An inspection of the table indicates, in the first place, that the total serum calcium is quite constant in the guinea pig even in cases of severe shock. However, much work has been done in recent years to show that the biological activity of calcium really depends upon the various forms in which it may exist. Since the discovery of Rona and Takahashi (9) that serum calcium exists in diffusible and non-diffusible form, much evidence has accumulated (10–12) to indicate that the non-diffusible fraction is in the form of a protein compound. This explanation has been rejected by Cameron and Moorhouse (13) and, though it is not the purpose of this paper to discuss the merits of these theories, we should like to point out, that, at present, it appears to be accepted that the non-diffusible serum calcium is in the form of a protein complex. Moreover, the total serum calcium determination

may be a poor index of what is really happening, for a disturbance of this calcium-protein complex may occur with resulting marked

TABLE I.

Blood Calcium Distribution in the Normal and Shocked Guinea Pig.

Experiment No.	Total Ca	Diffusible Ca		Non-diffusible Ca		Remarks
		Mg. per 100 cc.	Per cent of total	Mg. per 100 cc.	Per cent of total	
	mg. per 100 cc.					
1	10.2	5.4	53	4.5	44	Normal animals
2	8.6	4.6	54	4.0	47	Normal animals
3	10.2	5.9	58	4.0	44	Normal animals
4	8.6	4.7	55	3.6	42	Normal animals
5	9.6	6.5	67	3.2	33	Normal animals
6	10.1	5.3	52	5.0	49	Doubtful shock 20 min. after antigen injection
7	9.4	5.0	53	4.2	45	Negative shock. Bled 20 min. after injection
8	9.4	5.1	54	4.2	45	Doubtful shock. Bled 20 min. after injection
9	8.8	5.2	59	3.5	40	Negative shock. Passive sensitization
10	8.4	4.9	58	3.6	43	Maximum shock; bled at prostration
11	10.0	7.1	71	3.2	32	Maximum shock; bled at prostration
12	9.3	5.9	63	3.2	34	Maximum shock; bled at prostration
13	9.2	6.6	72	2.7	30	Maximum shock; bled at prostration
14	9.7	6.2	64	3.6	37	Maximum shock; bled at prostration; passive sensitization
15	9.3	6.7	72	3.1	33	Immediate shock; bled on appearance of clonic seizures; lungs maximum
16	9.6	5.7	59	4.1	43	Immediate shock; bled on appearance of clonic seizures; lungs maximum
17	10.1	7.3	72	3.0	30	Immediate shock; bled on appearance of clonic seizures; lungs maximum
18	10.0	7.3	73	2.7	27	Histamine, 1 cc. 1/5000 dilution; immediate violent shock; bled at prostration

physiological effects, but without altering the total serum calcium. A striking example of this has recently been pointed out by Shelling and

Maslow (14) who demonstrated that, following citrate additions to serum, either *in vitro* or *in vivo*, practically the entire calcium-protein combination is destroyed, all the calcium becoming diffusible, without, however, altering the total serum calcium. In order to determine if the reaction in the guinea pig following citrate injection resembled anaphylaxis, Shelling and Maslow's procedure was followed in one set of animals: calcium determinations (not listed in the tables) indicated that over 80 per cent of the guinea pig serum calcium became diffusible. Following the citrate injections violent immediate reactions were elicited; these, however, did not in any way simulate those characteristic of anaphylactic shock.

The findings for diffusible calcium, in terms of percentage of total calcium, are shown in the table to vary from 53 to 67 with an average of 55 for the normal animals; for the subsensitized which did not manifest typical symptoms upon reinfection, the variations were from 52 to 59 with an average of 54.5; while in the shocked animals a minimum value of 58 and a maximum of 72, with an average of 66, were found. Experiments 10 and 16 of the shocked animals gave values which may be considered "high" normal, while the results of the other experiments indicated a very definite increase in diffusible calcium with a corresponding decrease in the non-diffusible or "protein-complex" calcium.

There is then no change in this form of calcium incident to the treatment of the animal with a foreign serum, but a decided increase when the animals manifest shock. This increase is apparently not affected by the time interval at which the animal is bled, once symptoms of acute shock are established.

That the calcium change is not peculiar to anaphylactic shock is evidenced by the experiment with citrate injections and with histamine, resulting in the highest figure found for diffusible calcium. But in this last type of reaction, the coagulability of the blood is known not to be affected. This change in calcium ion content of the blood in anaphylactic shock is then not of necessity directly related to the phenomenon of a lessened coagulation time. This last has, indeed, been adequately accounted for by experiments of Weil (15) which demonstrated that the lessened coagulation time could be attributed to a liver product thrown into circulation following contact with the

antigen. From the results found for the non-diffusible fraction, it may be inferred that some change occurs in the protein of the serum whereby its normal combination with calcium is disturbed; but the data at hand furnish no basis for any conclusions as to the rôle this change plays in the basic mechanism of the phenomenon of shock.

CONCLUSION.

The results above reported for total calcium and the membrane-diffusible fraction in the serum of the guinea pig, taken at various intervals during anaphylactic shock, confirm the findings of previous workers that the total calcium is essentially unchanged. There is, however, the further finding that the diffusible fraction is considerably increased over that found for the animal similarly treated but not manifesting characteristic symptoms.

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