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ON THE PRESENCE OF "NOVADRENINE" IN SUPRARENAL EXTRACTS.

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Recently Szent-Györgyi and his co-workers [Annau, St Huszák, Svirbely and Szent-Györgyi, 1932] reported in this *Journal* on observations revealing a discrepancy between colorimetrical and physiological adrenaline tests in extracts from bovine suprarenal medulla, which they thought to be explained most adequately by the supposition of a new and more active form of adrenaline. For this substance the investigators have proposed the name "Novadrenine."

The assumption that the effects observed cannot be attributed to adrenaline is based chiefly on the following observations:

(1) The adrenaline content of the bovine suprarenal medulla was found colorimetrically to be 1–2 mg. per g., whether the physiological activity of the extracts was high or not.

(2) The physiological activity of highly active extracts from the suprarenal medulla was found to correspond to 15–30 mg. of adrenaline per g. of tissue, or 10–20 p.c. of the dry weight, which is considered impossible.

The striking results obtained by Szent-Györgyi and his co-workers made it desirable to study the question of "novadrenine" further. The following experiments were carried out in the course of an attempt to work out a convenient method for estimating adrenaline colorimetrically. The adrenaline content of the suprarenal medulla from oxen has been studied in a number of experiments, the conditions under which Szent-Györgyi and his co-workers were able to obtain their active substance having been followed closely. One of the experiments, all of which gave the same results, will be communicated in detail below.

Experimental.

Immediately after death the suprarenals from an ox (weight 710 kg.) were prepared and put in a glass vessel in a Dewar's flask with cold mixture (– 6° C.). The glands arrived at the laboratory about ¾ hour after the death of the animal. The preparation was then proceeded with immediately. One gland was frozen by means of CO_2 and cut into slices of about 0·2 mm. in thickness, after removal of the cortex. The slices of medulla, weighing 4·5 g., were kept frozen until they were suspended in 9 c.c. of 0·5 p.c. trichloracetic acid, that is 2 c.c. for each gram of pulp, as done by Szent-Györgyi and his co-workers. After thorough mixing the extract was rapidly heated to 80° C. and quickly cooled down again with the aid of iced water. The mixture was then filtered and kept in the ice-box (temperature – 5° C.). The filtered solution was slightly opaque, but after standing in the ice-box for about an hour it passed through the filter quite clear.

The second gland of the pair after arrival at the laboratory was kept at room temperature. The preparation of the first gland having been completed, the medulla of the second one was separated by means of a scalpel. 3·6 g. of the medulla were then ground up in a mortar with *N*/10 HCl and fine sand, following the procedure of Folin, Cannon and Denis [1912–13]. After a short boiling, the addition of sodium acetate and reboiling, the extract was cooled down and filtered, giving a clear filtrate.

The adrenaline contents of the extracts were then immediately estimated biologically and colorimetrically.

In order to determine the physiologically active adrenaline the blood-pressure method was employed. The determinations were made on cats under ether and on rabbits anæsthetized with urethane. The standard used was Supraren. synth. cryst. pur. (Höchst). Both kinds of test animals gave the same results. Part of the tracings from one experiment with suprarenal extracts on the blood-pressure of a rabbit under urethane is given in Fig. 1.

For comparison the following solutions were used:

A. Medullary extract according to Folin and his co-workers; 1 c.c. extract corresponds to 0·8 mg. of the medulla.

B. Medullary extract according to Szent-Györgyi and his co-workers; 1 c.c. extract corresponds to 0·6 mg. of the medulla.

Standard, 0·005 mg. adrenaline per c.c.

All extracts were made up with saline, which was adjusted to a slightly acid reaction to litmus by means of HCl.

From the biological determinations it was found that: 1 c.c. *A* corresponded in action to 0·0114 mg. adrenaline, 1 c.c. *B* corresponded in action to 0·0078 mg. adrenaline, if 2·5 γ adrenaline = 0·22 *A* = 0·32 *B*.

From these figures the adrenaline content of the medulla would be: in the gland prepared *ad A* 14·3 mg. adrenaline per g. fresh medulla, and in the gland prepared *ad B* 13·0 mg. adrenaline per g. of fresh medulla.

Apparently the two methods used give about the same yield of adrenaline. The figures obtained from the experiment communicated

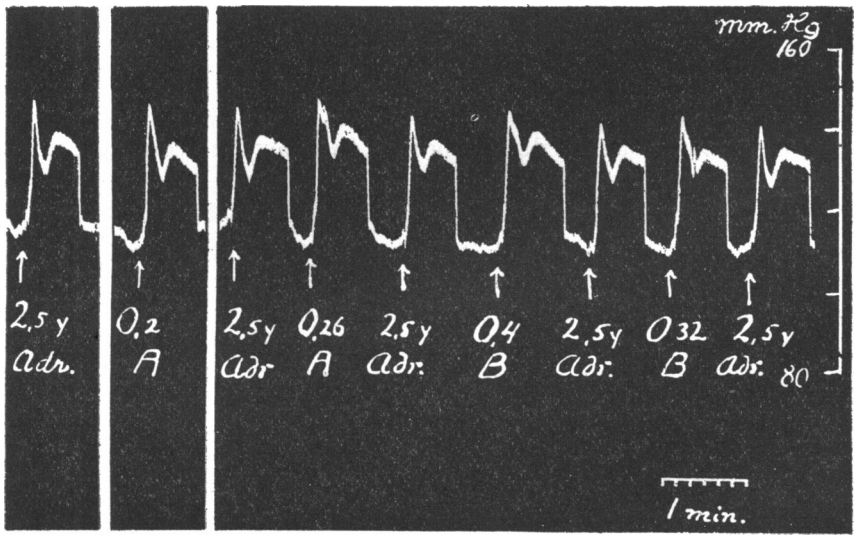

Fig. 1. Rabbit blood-pressure under urethane anæsthesia. 0·22 c.c. *A* (extract from bovine suprarenal medulla according to Folin) corresponds in action to 0·32 c.c. *B* (medullary extract according to Szent-Györgyi) and to 0·0025 mg. adrenaline.

thus agree fairly well with those found by Szent-Györgyi and his co-workers in active extracts, and also with those communicated in the recent review by Kojima, Nemoto, Saito, Sato and Suzuki [1932] with extracts from bovine suprarenal medulla.

The colorimetrical determinations were carried out by a spectrophotometric method recently worked out which is based on the Vulpian [1856] reaction [Euler, 1933]. This method was found to give figures in fairly close agreement with those found biologically.

The instrument used was a Pulfrich "Stuphenphotometer" (Zeiss). The following figures were obtained by testing the extracts about 1 hour

after the commencement of the preparation. The extracts were 10 times as strong as those used in the rabbit blood-pressure experiment (extract *a* thus = 10 *A*, *b* = 10 *B*).

Extract	Adrenaline found colorimetrically
1 c.c. *a*	0·118 mg.
1 c.c. *b*	0·081 mg.

The amounts of adrenaline per g. of medulla will, from these determinations, amount to 14·7 mg. and 13·5 mg. per g. respectively.

The agreement between the two estimates, colorimetric and biological, hardly admits of the assumption of a special, more highly active adrenaline-like substance in this case, though the physiological activity of the extracts was of the same order as in the active extracts of Szent-Györgyi and his co-workers.

Further, the two methods of extraction gave practically the same amount of adrenaline as calculated per g. of medulla, though no precautions were taken as regards cooling etc. in the case of the Folin extract.

Other extracts were prepared after freezing the glands immediately after their arrival at the laboratory by means of liquid air. No significant difference could be detected, however, as to the adrenaline content as determined either colorimetrically or biologically.

It might thus be assumed that—as significant differences between the animals used in the experiments would hardly be the cause of the discrepancy—the colorimetric method used by Szent-Györgyi and his co-workers is less suitable. This suggestion is supported by the fact that the physiologically determined activities are of the same order in active extracts, though the colorimetrical determinations differ.

The colorimetric method used by Szent-Györgyi and his co-workers is also based on the Vulpian reaction. The extracts were neutralized with a small excess of sodium bicarbonate. Even in slight alkaline solution, however, the colour is liable to fade away rather quickly, as I have had the opportunity of observing, so that, after a short time, the activity would appear less than the original. Szent-Györgyi and his co-workers note that the colour was stable a minute or so. It may be assumed, however, that the fading starts before the determination has been completed, thus giving unreliable results, unless the value at zero time has been calculated from the fading velocity in each case.

SUMMARY.

Extracts from the bovine suprarenal medulla have been prepared according to Szent-Györgyi and his co-workers, and their activity determined physiologically and colorimetrically.

No significant difference was observed between the activity as found by biological tests (rabbit blood-pressure) and that found colorimetrically (using the method of Euler).

An attempt to explain the different results is made[1].

REFERENCES.

Annau, E., St Huszák, Svirbely, J. L. and Szent-Györgyi, A. (1932). *J. Physiol.* **76**, 181.

Euler, U. S. v. (1933). *Biochem. Z.* **260**, 18.

Folin, O., Cannon, W. B. and Denis, W. (1912–13). *Biochem. J.* **13**, 477.

Kojima, T., Nemoto, M., Saito, S., Sato, H. and Suzuki, T. (1932). *Tohoku J. exp. Med.* **19**, 205.

Vulpian, A. (1856). *C. R. Acad. Sci. Paris*, **43**, 663.

[1] This paper was kindly submitted by Dr U. S. v. Euler for my criticism. v. Euler is able to give an adequate explanation of the experiments of my collaborators and myself, without the necessity of supposing the existence of a more active form of adrenaline (novadrenine). I accept his view, by which the supposition of such a compound becomes superfluous.—A. Szent-Györgyi.