On the Existence of So-called "Virtual Adrenaline" of the Suprarenal Gland.

By

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Existence of "virtual adrenaline", a parent substance of adrenaline, or the combined adrenaline in the suprarenal gland besides the adrenaline or the free adrenaline was first claimed in France¹⁾ and has been much studied and discussed there²⁻¹⁴⁾, just as the case of the sucre virtuel.

Not only has its existence been seriously questioned by several writers¹⁵⁻¹⁷⁾, but there are obviously some evident contraversies among the data given by the French investigators. The one sees the site of production of the virtual adrenaline in the cortex^{6) 9) 12) 14)}, but the other in the medulla^{8) 10) 11) 13)}, for example.

- 1) Abelous, Soulié and Toujan, Soc. Biol., 1905, **58**, 533.
- 2) Mouriquand and Leulier, C. r., 1926, **183**, 1353.
- 3) Leulier and Gojon, Soc. Biol., 1927, **96**, 547.
- 4) Paget and Lohéac, J. de Pharmacie et de Chim., 8 ser., 1928, **8**, 159.
- 5) Langeron, Paget and Lohéac, Soc. Biol., 1928, **98**, 1424.
- 6) Abelous and Argaud, Soc. Biol., 1929, **102**, 893.
- 7) Ishibashi, Hokuetsu Igakkai Zasshi, 1930, 45 y., 390.
- 8) Leulier and Revol, C. r. 1930, **190**, 452.
- 9) Abelous and Argaud, C. r., 1931, **193**, 369.
- 10) Paget, J. de Pharmacie et de Chim., 8 ser., 1931, **13**, 617.
- 11) Leulier and Revol, Bull. Soc. Chim. Biol., 1931, **13**, 211.
- 12) Abelous and Argaud, C. r., 1932, **195**, 338.
- 13) Mouriquand, Leulier and Revol, Rev. franc. d' Endocrinol., 1933, **11**, 324.
- 14) Abelous and Argaud, C. r., 1934, **199**, 318.
- 15) Molinelli and Mazzocco, Soc. Biol., 1928, **99**, 1001.
- 16) Binet and Weller Soc. Biol., 1934, **115**, 598.
- 17) Devine, Biochem. J., 1936, **32**, 1768.

The present writer wishes to contribute a small experience towards this question under confusion. The adrenaline content was determined by the colorimetical method of Suto-Kojima¹⁸⁾. The gland was powdered by liquid air, and extracted and determined for adrenaline by adding the saturated sublimate solution containing acetic acid and then warming it in the water bath of 70-80°.

The Bailly method¹⁹⁾, which was used by the French investigators except Abelous, is similar to that of Comessatti and of Suto-Kojima, as the above description shows. Of the method of Bailly, Fabre said of its concordance with the biological assay in respect to the value of adrenaline obtainable by their use²⁰⁾. That while the Bailly method yielded about the same results as Mouriquand the question of "virtual adrenaline", quite a different outcome was obtainable when the blood pressure method was applied, was the outcome of Mollinelli and Mazzocco²¹⁾. Further, that quite a different outcome is given according whether the extraction is made by means of trichloroacetic acid or sulphuric acid, was reported by Binet and Weller²²⁾. The determination was made there by iodine. The latter gave the data which accord with Mouriquand. The permanganate reaction gave in the hands of Devine²³⁾ an incomparatively low value of adrenaline in comparison with the cat blood pressure method. It was 1:4 in the cases of Devine, while it was 1:1.5 in this Laboratory²⁴⁾.

In the present experiments the glands of pigs were used; they were removed from half an hour to one hour after the animal was killed at the city slaughter house of Sendai. From 15 to 20 minutes thereafter, one of the pair glands was soon estimated on its adrenaline content by the Suto-Kojima method. The other was dried in a vacuum exciccator arranged upon sulphuric acid for 24 hours. Then, the adrenaline was determined. The actual concentration of adrenalin chloride solution of Sankyo Co. (1:1000), which was employed as the standard, was assayed by Kodama's modification of the colorimetical method of Folin and others²⁵⁾.

DATA.

In order to have, as the control, the ratio of the adrenaline content between the pair glands, the glands from 10 normal pigs were worked out without delay in the Laboratory with the data given in Table I.

18) T. Kojima, *Tohoku J. of Exp. Med.*, 1928, **10**, 281.

19) Bailly, *J. de Pharmacie et de Chim.*, 7 ser., 1924, **30**, 404.

20) Fabre, *Ibid.*, 8 ser., 1926, **4**, 84.

21) Hatanô, *Tohoku J. of Exp. Med.*, 1936, **29**, 314 ff.

22) Kodama, *J. of Biochem.*, 1922, **1**, 281.

(In all the tables "epinephrine" was used instead of adrenaline as usual.)

The left glands contained 2.93–7.66 mg., mean 4.36 mg. adrenaline, that is, 1.36–2.11 mg., mean 1.73 mg. per g. of gland, and the right gland 2.47–7.66 mg., mean 4.17 mg. in toto and 1.23–2.11 mg., mean 1.67 mg. per g. of gland. The values check well with those obtained by Kojima and others²³⁾.

The ratio of the adrenaline content between both glands was (l:r) 1:0.83–1:1.08, mean 1:0.95 in toto, and 1:0.84–1:1.13, mean 1:0.97 per g. of gland. The left gland contains a little more adrenaline than the right; as the mean the excess is 5 % in toto and 3 % per g. of gland.

The principal experiment is composed of two sets, and each set of

TABLE I.

Pig suprarenals. The whole gland.

Epinephrine content of the whole gland determined by means of Suto-Kojima's method.

No., sex of animal	Date of experiment	Approximate weight of body (kg.)	Treated with	Weight of suprarenal (g.)	Epinephrine content (mg.)		Ratio between sister glands (l:r)		
					total	per g. of gland	Weight	Epinephrine content	
								total	per g. of gland
4 ♀	26. XI. 1937	82.5	sand	{l. 1.90 r. 2.30	3.71 3.76	1.95 1.63	1:1.21	1:1.01	1:0.84
5 ♀	30. XI. "	82.5	liquid nitrogen	{l. 1.97 r. 1.67	2.93 2.81	1.49 1.68	1:0.85	1:0.96	1:1.13
7 ♀	6. XII. "	102.5	sand	{l. 2.77 r. 2.53	5.05 4.17	1.82 1.65	1:0.91	1:0.83	1:0.90
9 ♀	14. XII. "	67.5	sand	{l. 2.10 r. 2.01	2.98 2.47	1.42 1.23	1:0.96	1:0.83	1:0.87
11 ♀	22. XII. "	150	sand	{l. 5.11 r. 4.87	7.66 7.66	1.50 1.57	1:0.95	1:1	1:1.05
13 ♀	13. I. 1938	112.5	liquid nitrogen	{l. 2.46 r. 2.40	4.66 4.66	1.89 1.94	1:0.98	1:1	1:1.03
15 ♂	15. I. "	75	liquid nitrogen	{l. 2.39 r. 2.30	3.91 3.53	1.64 1.54	1:0.96	1:0.90	1:0.94
16 ♀	15. I. "	67.5	liquid nitrogen	{l. 2.31 r. 2.08	3.15 2.83	1.36 1.36	1:0.90	1:0.90	1:1
19 ♀	22. I. "	75	sand	{l. 2.03 r. 2.20	4.28 4.64	2.11 2.11	1:1.08	1:1.08	1:1
22 ♂	28. I. "	67.5	liquid nitrogen	{l. 2.51 r. 2.66	5.28 5.15	2.10 1.94	1:1.06	1:0.98	1:0.92
Mean					{l. 2.55 r. 2.50	4.36 4.17	1:0.99	1:0.95	1:0.97

23) Kojima, Nemoto, Saito, Sato and Suzuki, Tohoku J. of Exp. Med., 1932, 19, 205.

6 pairs of glands. In one set, the left gland was worked out immediately when it was brought to the Laboratory, and the right gland after 24 hours' staying in the vacuum exsiccator containing sulphuric acid, while the right gland was worked out immediately in the second set. The data are given in Tables II and III respectively.

The adrenaline content was estimated in the I set as: The left glands, which were treated soon, contained 3.62 mg. in toto (2.71-5.11 mg.), viz. 1.78 mg. (1.45-2.72 mg.) per g. of gland, and the right glands, which were dried for 24 hours in the vacuum exsiccator, contained 3.80 mg. (3.05-5.91 mg.) in toto and 1.99 mg. (1.54-3.13 mg.) per g. of gland. The ratio of adrenaline load in both glands (L:R) was therefore 1:1.05 (1:0.79-1:1.16) in the total amount, and 1:1.13 (1:0.95-1:1.34) in the value per unit of gland weight. If the ratios in the control experiments, 1:0.95 and 1:0.97 respectively, be taken into account, the adrenaline load in the right gland, that is, that gland which was left for 24 hours in the vacuum exsiccator, must be assumed as having increased by 10% in the total amount and by 16% when reckoned on the gland weight.

TABLE II.

Pig suprarenals. The whole gland.

Epinephrine content of the whole gland determined by means of
Suto-Kojima's method.

(Right suprarenals were dried in the vacuum exsiccator arranged upon sulphuric acid for 24 hours before the determination of epinephrine.)

No., sex of animal	Date of experiment	Approximate weight of body (kg.)	Treated with	Weight of suprarenal (g.)	Epinephrine content (mg.)		Ratio between sister glands (l:r)		
					total	per g. of gland	Weight	Epinephrine content	
								total	per g. of gland
3 ♂	26. XI. 1937	78.8	sand	{l. 2.28 r. 1.76	3.30 3.40	1.45 1.93	1:0.77	1:1.03	1:1.34
6 ♂	30. XI. "	75		{l. 2.05 r. 1.82	3.50 3.71	1.71 2.04	1:0.89	1:1.06	1:1.19
8 ♀	6. XII. "	82.5		{l. 1.82 r. 1.92	2.71 3.09	1.49 1.61	1:1.05	1:1.14	1:1.08
10 ♀	14. XII. "	67.5		{l. 2.12 r. 1.75	3.88 3.05	1.83 1.74	1:0.83	1:0.79	1:0.95
12 ♀	22. XII. "	82.5		{l. 1.88 r. 1.89	5.11 5.91	2.72 3.13	1:1.01	1:1.16	1:1.15
14 ♂	13. I. 1938	75	"	{l. 2.17 r. 2.38	3.21 3.66	1.48 1.54	1:1.10	1:1.14	1:1.04
Mean				{l. 2.05 r. 1.92	3.62 3.80	1.78 1.99	1:0.94	1:1.05	1:1.13

TABLE III.

Pig suprarenals. The whole gland.

Epinephrine content of the whole gland determined by means of
Suto-Kojima's method.

(Left suprarenals were dried in the vacuum exsiccator arranged upon sulphuric acid for 24 hours before the determination of epinephrine.)

No., sex of animal	Date of experiment	Approximate weight of body (kg.)	Treated with	Weight of suprarenal (g.)	Epinephrine content (mg.)		Ratio between sister glands (l:r)		
					total	per g. of gland	Weight	Epinephrine content	
								total	per g. of gland
17 ♂	18. I. 1938	75	sand	{l. 1.69 r. 1.62	4.26 4.55	2.52 2.81	1:0.96	1:1.07	1:1.12
18 ♂	"	93.8	"	{l. 2.11 r. 2.13	3.72 3.28	1.76 1.54	1:1.01	1:0.88	1:0.87
20 ♂	28. I.	67.5	"	{l. 1.79 r. 1.84	4.62 4.49	2.58 2.44	1:1.03	1:0.97	1:0.95
21 ♀	"	75	"	{l. 2.82 r. 2.50	6.68 6.08	2.37 2.43	1:0.89	1:0.91	1:1.03
25 ♀	12. II.	75	"	{l. 2.63 r. 2.75	6.90 6.60	2.62 2.40	1:1.05	1:0.96	1:0.92
26 ♂	"	67.5	"	{l. 2.39 r. 2.59	6.53 5.60	2.73 1.93	1:1.06	1:0.77	1:0.71
Mean				{l. 2.24 r. 2.24	5.45 5.00	2.43 2.26	1:1	1:0.93	1:0.93

In the second set, the left gland was reserved for 24 hours before the determination in the exsiccator. The figures are:

Left: 5.45 mg. (3.72–6.92 mg.) in toto; 2.43 mg. (1.76–2.73 mg.) per g. of gland.

Right: 5.00 mg. (3.28–6.60 mg.) in toto; 2.26 mg. (1.54–2.81 mg.) per g. of gland.

Ratio (L:R) 1:0.93 (1:0.77–1:1.07) in "toto"; 1:0.93 (1:0.71–1:1.12) in the value reckoned on the body weight.

If these ratios in the average values are converted into those of the right gland to the left, they are 1:1.08 in the total amount as well as in the value reckoned on the body weight. The ratios of the values in the control experiments, if reckoned in the same manner, are (R:L) 1:1.05 and 1:1.03 respectively. The left gland increased therefore its adrenaline content by the manipulation by 3% and by 5% respectively.

In the first set 5 cases out of 6 showed an increase of the adrenaline load by staying in vacuum for 24 hours, but, half of the cases in the second set had a similar tendency, while in 2 cases the load was apt rather to decrease, and in the remaining single case no change was found. This non-occurrence of

the increase might be due to the insufficiency of drying, which was due in turn to the reduction of the intensity of sulphuric acid. The experiments on the glands of pigs, Nos. 25 & 26, were carried out after the sulphuric acid was newly changed, then the increase was definite.

All the data being taken altogether, it may be said that the adrenaline content in the suprarenal capsule increased in the majority of cases when kept for 24 hours in the vacuum exciccator at the room temperature; the value determined at once was taken as the standard. The excess was about ten per cent. The excess did not exceed usually twenty per cent, only in a single case it was thirty per cent.

This magnitude of increase in the present investigation, which is small, corresponds well with that of Ishibashi on rabbits, but is very different from the figures reported by some French writers. In the paper of Molinelli there are also a very large increase, such as three times as much.

Further there is a very remarkable difference between the figures in their papers and those in the present. Namely, those in the previous papers are full of very large variations, while those in our cases are pretty uniform.

SUMMARY.

One of the pair suprarenals of pigs was estimated of the adrenaline content as soon as possible by the Suto-Kojima method, and the other was left for 24 hours in the vacuum exciccator arranged upon sulphuric acid, and then assayed.

In the majority of cases the letting stand in the above manner effected to increase the adrenaline content by about ten per cent as the mean; this figure is of quite an another order than that reported by Mouriquand and others.