The Content of Noradrenaline and Adrenaline in the Rat Heart After Administration of Rauwolfia Alkaloids[1]

Reserpine has been shown to lower the catechol amine content of brain, peripheral sympathetic tissues, adrenal medulla, heart, and artery walls[2–10]. The heart rate increase produced by reserpine in the dog heart-lung preparation has been ascribed by PAASONEN and KRAYER[9] to the noradrenaline released from the heart tissue itself. In connection with the experiments described in the last mentioned publication the effect of a number of Rauwolfia alkaloids on the adrenaline and noradrenaline content of the rat heart was determined.

The assays of the noradrenaline and adrenaline content were made in the heart taken from intact rats. Two hearts were combined for each analysis. The tissues were ground in 5% trichloroacetic acid and the procedure was that described by V. EULER[11], except for minor changes[9]. The samples were analyzed biologically by using the cat blood pressure and the isolated rectal caecum of the hen. Before starting the blood pressure assay a combination of drugs, as used by KÄRKI[12], was administered. The methods and materials have been described by PAASONEN and KRAYER[9].

The Rauwolfia alkaloids (generously supplied by S. B. Penick & Co., N. Y.) were dissolved in glacial acetic acid and the solution diluted so that the concentration of acetic acid injected was not in excess of 2%. The drugs were injected intraperitoneally. The doses of L-noradrenaline monohydrate and L-adrenaline bitartrate (Sterling-Winthrop) are given in terms of the base.

Results. The reserpine-type alkaloids[13], raunescine, isoraunescine, deserpidine (canescine, recanescine), and rescinnamine, upon intraperitoneal injection, produced a marked depletion of noradrenaline in the rat heart at the dose level of 5 mg/kg. The data are assembled in the Table. The intensity of the effect is similar to that observed with reserpine at the same dose level[9]. Even at a level of 20 mg/kg, yohimbine, which is chemically different from the reserpine-type group, had no influence upon the noradrenaline content of the heart.

For a quantitative comparison of the ability of different reserpine-type alkaloids to release noradrenaline, the dose of 5 mg/kg appears to be too high. A clear-cut difference in potency between raunescine and isoraunescine was evident at the dose of 0·5 mg/kg. At this dose, isoraunescine removed only about 50% of the noradrenaline within 6 h, while raunescine was fully as active as at the ten times higher dose. In four experiments, not included in the Table, 0·5 mg/kg of reserpine, within 2 h, removed 69% (range 58–77) of the noradrenaline content of the heart.

Effect of Rauwolfia Alkaloids on the Noradrenaline and Adrenaline Content of the Rat Heart

Substance	Dose (mg/kg)	Time[a] (h)	Individual Values mμg/g[b]	Mean mμg/g[c]
			Noradrenaline	
Control . . .			(n = 13)	490 ± 140[d]
Isoraunescine	0·5	6	245,250	248
Isoraunescine	5·0	4	68	68
Isoraunescine	5·0	6	28	28
Isoraunescine	5·0	16	<20[e],13	<17
Raunescine .	0·5	6	32,36	34
Raunescine .	5·0	4	34,15	25
Raunescine .	5·0	6	<28[e]	<28
Raunescine .	5·0	16	15,28	22
Deserpidine[f] .	5·0	4	71,30	51
Rescinnamine	5·0	4	17	17
Rescinnamine	5·0	6	<29[e]	<29
Yohimbine .	5·0	6	350,495	423
Yohimbine .	20·0	4	355,550	453
			Adrenaline	
Controls . .			(n = 10)	32 ± 14[d]
Isoraunescine	0·5	6	42,36	39
Isoraunescine	5·0	4	45	45
Isoraunescine	5·0	6	14	14
Isoraunescine	5·0	16	6,20	13
Raunescine .	0·5	6	26,39	33
Raunescine .	5·0	4	30,27	29
Raunescine .	5·0	6	14	14
Raunescine .	5·0	16	24,24	24
Deserpidine[f] .	5·0	4	28,30	29
Rescinnamine	5·0	4	45	45
Rescinnamine	5·0	6	15	15
Yohimbine .	5·0	6	34,41	38

[a] Time after intraperitoneal injections.
[b] Each individual value is obtained from two pooled hearts.
[c] Wet weight.
[d] ± standard deviation.
[e] (<) Not detectable and less than the value presented.
[f] Also called canescine or recanescine.

Adrenaline amounted to about 6% of the noradrenaline content. The scatter of the adrenaline values was rather wide and the administration of the Rauwolfia alkaloids did not produce clear-cut changes.

In the experiments of INNES, KRAYER, and WAUD[14] the reserpine-type Rauwolfia alkaloids increased the heart rate and shortened the functional refractory period of atrioventricular (A–V) transmission and A–V propagation time in the dog heart-lung preparation. The quantitative difference in potency between Raunescine and Isoraunescine, mentioned above, was also noticed. α-Yohimbine which had no such effects did not decrease the noradrenaline content in our experiments. The assumption therefore, that the transient cardioacceleration and facilitation of A–V transmission is due to the release of noradrenaline from the heart, is further supported by our findings on the rat heart.

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[2] M. HOLZBAUER and M. VOGT, J. Neurochem. *1*, 8 (1956).

[3] E. MUSCHOLL and M. VOGT, J. Physiol. *141*, 132 (1958).

[4] A. CARLSSON and N.-Å. HILLARP, Kungl. fysiogr. Sällsk. Förh. Lund *26*, 90 (1956).

[5] Å. BERTLER, A. CARLSSON, and E. ROSENGREN, Naturwissenschaften *43*, 521 (1956).

[6] A. CARLSSON, E. ROSENGREN, Å. BERTLER, and J. NILSSON, in *Psychotropic Drugs* (Ed. by GARATTINI and GHETTI, Elsevier, Amsterdam 1957), p. 363.

[7] O. KRAYER and M. K. PAASONEN, Acta physiol. scand. *42*, Suppl. *145*, 88 (1957).

[8] M. K. PAASONEN and O. KRAYER, Fed. Proc. *16*, 326 (1957).

[9] M. K. PAASONEN and O. KRAYER, J. Pharmacol. *123*, 153 (1958).

[10] J. H. BURN and M. J. RAND, Brit. med. J. *1*, 903 (1958).

[11] U. S. v. EULER, *Noradrenaline* (C. C. Thomas, Springfield, Ill. 1956), p. 64.

[12] N. T. KÄRKI, Acta physiol. scand. *39*, Suppl. *132*, 1 (1956).

[13] N. J. BEIN, Pharmacol. Rev. *8*, 435 (1956).

[14] I. R. INNES, O. KRAYER, and D. R. WAUD, J. Pharmacol., in press (1958).

According to BRODIE *et al.*[15,16] only Rauwolfia alkaloids with sedative action are able to release 5-hydroxytryptamine and noradrenaline from brain tissue. Isoraunescine in our experiments was practically without sedative action but, nevertheless, able to deplete noradrenaline from the rat heart. However, a higher dose of isoraunescine than raunescine seemed necessary to produce the effect. In the brain, too, 50 mg/kg of isoraunescine lowered the concentration of noradrenaline less than 5 mg/kg of raunescine[17]. The fact that α-yohimbine was ineffective in the experiments reported is in keeping with the finding[18,19] that it does not release noradrenaline from the rat intestine, the tissue most sensitive to depletion of noradrenaline by the reserpine-type Rauwolfia alkaloids.

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Zusammenfassung

Die chemisch verwandten Rauwolfia-Alkaloide Raunescin, Isoraunescin, Deserpidin, Rescinnamin und Reserpin verursachen einen starken Abfall des Noradrenalingehaltes des Rattenherzens, wenn sie intraperitoneal in einer Dosis von 5 mg/kg gegeben werden. Der Adrenalingehalt wird nicht deutlich oder einheitlich verändert. α-Yohimbin, das chemisch zu einer andern Gruppe gehört, hat keine Wirkung auf den Noradrenalingehalt des Herzens.

[15] B. B. BRODIE, P. A. SHORE, and A. PLETSCHER, Science *123*, 992 (1956).

[16] P. A. SHORE and B. B. BRODIE, in *Psychotropic Drugs* (Ed. by GARATTINI and GHETTI, Elsevier, Amsterdam 1957), p. 442.

[17] M. K. PAASONEN and P. B. DEWS, Brit. J. Pharmacol. *13*, 84 (1958).

[18] M. K. PAASONEN and N. T. KÄRKI, Acta endocr., in press (1958).

[19] N. T. KÄRKI and M. K. PAASONEN, J. Neurochemistry, in press (1958).

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Effects of Different Vegetable Fats on the Cardiac Damage Caused by Pyridoxine Deficiency

Hypertrophy of the heart[1] and a reduction in cardiac transamination[2] are exhibited by animals when vitamin B_6 is lacking from their diet, the former being attributed to hypertension caused by lack of pyridoxine, while the latter is basically due to the vitamin in the form of pyridoxalphosphate being the prosthetic group of transaminases.

Such a picture is remarkably similar to the one observed in human arteriosclerotic forms, whose pathogenesis appears to be heavily affected by vitamin B_6[3] through the relations existing between the latter and lipid metabolism, notably that of the so-called essential fatty acids. It has been established, indeed, that, while a lack of vitamin B_6 tends to increase the severity of the Burr syndrome[4], the administration of non-saturated fatty acids during a diet lacking in pyridoxine will either delay the onset of the hypovitaminosis or mitigate its symptomatology[5].

Table I.—Iodine Values of Fats used (Mean values ± S.E.)

Fat	Iodine Value
Coconut Oil	10·2 ± 0·4
Cocoa Butter	36·5 ± 2·1
Olive Oil	81·9 ± 3·4
Peanut Oil	93·8 ± 6·0
Cottonseed Oil	105·3 ± 8·8
Sesame Oil	107·1 ± 9·2
Corn Oil	123·8 ± 11·5
Wheat Germ Oil	125·2 ± 10·8
Soybean Oil	132·3 ± 12·1
Linseed Oil	177·6 ± 13·4

We were induced by the foregoing considerations to undertake a program aimed to assess the actions exerted by a variety of vegetable fats, all with varying contents of essential fatty acids, upon (1) the size, (2) lipid contents, and (3) pyridoxal phosphate contents of the heart, our experiments being performed on rats submitted to a diet lacking in vitamin B_6.

We used a total of 144 animals averaging in weight from 120 to 150 g, divided into twelve groups of twelve subjects each, on a diet with no vitamin B_6, of known composition to which 25% of a vegetable fat had been added.

The subjects received as much of the above diet as they could eat during a four-week period, at the end of which they were bled to death, their hearts then being removed under aseptic conditions, weighed and tested for total-lipids[6] and pyridoxalphosphate contents. The latter substance was determined by the method developed by BOXER *et al.*[7] using *S. faecalis* as the test germ.

The iodine value of each fat used was determined by the Hanus method as described in U.S.P. XV[8].

Our results are tabulated in Tables I and II.

From the foregoing data it appears that the greatest degree of heart enlargement is obtained from cocoa butter and coconut oil, the slightest from soybean and linseed oils, and that intermediate degrees of hypertrophy are determined by the other fats tested. It may be noted that the order in which the fats are classed according to their iodine values has been maintained, as the lower the iodine value of the fat administered, the greater was the increase in heart weight, and *vice versa*, the only exception being cottonseed oil, whose hypertrophying effect appears to be less than that of corn oil although its iodine value is lower.

Generally, the same considerations apply to the lipid contents of the heart tissue.

On the contrary, the pyridoxalphosphate contents was highest in such subjects as were administered soybean or wheat germ oil and lowest when olive oil was added to the diet. Starting from cottonseed oil, indeed, the effect upon the coenzymic contents of the heart tissue gradually

[1] L. R. C. AGNEW, Proc. Soc. exp. Biol. Med., N. Y. *90*, 452 (1955).

[2] S. R. AMES, P. S. SARMA, and C. A. ELVEHYEM, J. biol. Chem. *167*, 135 (1947).

[3] H. A. SCHROEDER, J. chron. Dis. *2*, 28 (1955).

[4] P. GYORGY and R. E. ECKARDT, Nature *144*, 512 (1939).

[5] T. W. BIRCH and P. GYORGY, J. biol. Chem. *131*, 761 (1939).

[6] W. O. FENN, J. biol. Chem. *128*, 297 (1939).

[7] G. E. BOXER, M. P. PRUSS, and R. S. GOODHART, J. Nutr. *63*, 623 (1957).

[8] U. S. Pharmacopoeia XV (Mack Publ. Co., Easton 1955), p. 896.