JCI The Journal of Clinical Investigation

URINARY OUTPUT OF ADRENALINE, NORADRENALINE, AND 3-METHOXY-4-HYDROXYMANDELIC ACID FOLLOWING CENTRIFUGATION AND ANTICIPATION OF CENTRIFUGATION

McC. Goodall, M. L. Berman

J Clin Invest. 1960;39(10):1533-1538. https://doi.org/10.1172/JCI104173.

Research Article

URINARY OUTPUT OF ADRENALINE, NORADRENALINE, AND 3–METHOXY–4–HYDROXYMANDELIC ACID FOLLOWING CENTRIFUGATION AND ANTICIPATION OF CENTRIFUGATION *

By McC. GOODALL and M. L. BERMAN

(From the Research Center and Hospital, University of Tennessee, Knoxville, Tenn., and the Acceleration Section, Biophysics Branch, Aerospace Medical Laboratory, Wright Air Development Division, Wright-Patterson Air Force Base, Ohio)

(Submitted for publication April 20, 1960; accepted June 9, 1960)

It has been shown that various forms of stress, i.e., thermal burn (1), muscular exercise (2), centrifugation (3–5), trauma (6) and so forth activate the sympatho-adrenal system and that this activation is manifested by an increase in the urinary output of adrenaline and noradrenaline. Furthermore, it has been demonstrated in the normal human that 3-methoxy-4-hydroxymandelic acid is one of the principal metabolic products of both adrenaline and noradrenaline (7–10). Hitherto, there have been no stress experiments correlating the urinary output of adrenaline and noradrenaline with that of 3-methoxy-4-hydroxymandelic acid. The experiments herein described not only demonstrate such a correlation but also show the effect of centrifugation and anticipation of centrifugation upon the urinary output of adrenaline, noradrenaline, and 3-methoxy-4-hydroxymandelic acid.

METHOD

A. Centrifugation

Nine healthy adult males were centrifuged on three different days to a 12 G forward acceleration to subjective tolerance. The ride did not exceed 3 minutes' duration and the rate of onset was 1 G per 5 seconds. During the time of centrifugation, the subjects in the free-swinging end of the WADD/human centrifuge were supported by a contoured net reclining seat (11). With this particular restraint system, subjects in a real centrifugation (12 G) faced the center of rotation with the head and spine forming an angle of 78° with the acceleration vector, the thighs and trunk forming an angle of 90° and the knees an angle of approximately 90°.

In order to evaluate the effect of anticipation upon the sympatho-adrenal system, some of the subjects were given mock centrifuge rides. The mock centrifugations were identical in all respects with those of a real centrifugation, except that subjects were spun at 2 rpm, equivalent to 0.02 G acceleration, for 3 minutes. However, to insure that no individual would be cognizant as to whether he was or was not to be centrifuged, the following random distribution of mock rides was instigated. The mock rides among the 9 subjects were such that 2 subjects had 3 real centrifugations, 2 subjects had 2 mock and 1 real centrifugation, and 5 subjects had 1 mock and 2 real accelerations, for a total of 18 actual and 9 mock centrifugations. As a further precaution to insure that the mock accelerations were unknown, subjects were not permitted to discuss their centrifuge rides.

B. Urine collections

During the 24 hours prior to experimentation, subjects were requested to refrain from ingesting coffee, tea, fruits, and all foods containing vanilla. This was done in order to avoid the accumulation of phenolic acids in the urine which might interfere with the detection of 3-methoxy-4-hydroxymandelic acid.

All subjects voided 2 hours before centrifugation and the urine was discarded. Thereafter, samples were collected approximately 1 hour before and 10 minutes before centrifugation; post-run samples were collected at approximately 30 minutes and 1 hour after centrifugation. Aliquots were preserved with a crystal of thymol and stored at − 10° C. In order to insure an adequate volume of urine, subjects drank 500 ml of water immediately after voiding the 1-hour pre-acceleration sample.

All control urine samples were obtained 3 weeks following experimentation. The urine was collected for 2 to 3 hours and at approximately the same time of day on which the subjects were originally centrifuged. All urine samples were assayed for adrenaline, noradrenaline, and 3-methoxy-4-hydroxymandelic acid (see paragraphs C and D, following).

C. Urinary adrenaline and noradrenaline

a) Preparation of urine extract. The procedure was adopted from von Euler and Hellner (12). The urine was hydrolyzed and the adrenaline and noradrenaline selectively adsorbed on aluminum hydroxide and filtered. The precipitate was washed and redissolved with 2 N sulphuric acid. The remaining salts were precipitated out by mixing the extract with alcohol and acetone. The filtrate was concentrated *in vacuo*.

* Supported by United States Air Force Contract AF-33-(616)-5397.

TABLE I

Average rate of urinary excretion of adrenaline in response to real and mock centrifugation in micrograms per hour

	Control	Time in minutes				
		−74	−11	0	+28	+89
Real centrifugation	0.86	1.03	1.38	Centrifugation	2.27	1.33
SD	±0.25	±0.09	±0.54		±1.02	±0.44
p*		<0.4	<0.01		<0.001	<0.01
Mock centrifugation	0.86	1.02	1.30		1.93	1.06
SD	±0.25	±0.61	±0.67		±0.77	±0.09
p*		<0.8	<0.05		<0.01	<0.2

* Comparison of experimental with control.

b) Bioassay of urine for adrenaline and noradrenaline. This procedure has been previously described (1, 12). In brief, the cat's blood pressure, which is sensitive to noradrenaline, is used in conjunction with the fowl's rectal cecum, which is sensitive to adrenaline. The cat's blood pressure was recorded from the carotid artery and injections of adrenaline, noradrenaline, and urinary extract were made into the femoral vein. The fowl's rectal cecum was suspended in a water bath containing Tyrode's solution at 39° C. Through the Tyrode's solution, 6.5 per cent of CO_2 in oxygen was bubbled. Injections of adrenaline, noradrenaline, and urinary extract were made into the bath and the degree of cecal relaxation was recorded on a kymograph.

c) Computation of results. Having determined the activity ratio for adrenaline and noradrenaline on the cat's blood pressure and on the hen's rectal cecum, and the activity of the unknown urinary extract in terms of L-noradrenaline, it is possible to calculate the relative amounts of adrenaline and noradrenaline in the urinary extract (1, 12).

D. Urinary 3-methoxy-4-hydroxymandelic acid

a) General. Urine samples, collected as outlined above, were extracted and the 3-methoxy-4-hydroxymandelic acid present was identified by two-dimensional paper partition chromatography. Quantitative estimation of 3-methoxy-4-hydroxymandelic acid was determined spectrophotometrically.

b) Preparation of urine extract. The method used was adopted from Armstrong (13) as modified by Berman and Pettitt (14). In brief, an aliquot of a measured volume of urine sample was acidified with 3 N HCl and extracted in ethyl acetate. After evaporation of the ethyl acetate, the residue was redissolved in absolute ethanol. The ethanol extract (0.5 ml) was applied to the filter paper with a specially designed semimicro automatic constant rate infusion pump and chromatographed in a two-phase solvent system. Three-methoxy-4-hydroxymandelic acid was visualized by spraying the chromatogram with a freshly prepared solution of diazotized *p*-nitroaniline.

c) Quantitative analysis of urinary 3-methoxy-4-hydroxymandelic acid. The details of this procedure were described by Berman and Pettitt (14). In brief, chromatographic areas containing the unknown were compared with known concentrations of 3-methoxy-4-hydroxymandelic acid. Areas containing unknown, known, and blanks of corresponding size were cut from the same chromatogram and eluted with an alcohol-sodium carbonate solution. The extinction coefficients for the eluates were determined against water at 520 mμ with a spectrophotometer. After subtraction of the blank values, a graph of the extinction coefficients against their concentration was made. The amounts of 3-methoxy-4-hydroxymandelic acid were calculated from this graph. Reproducibility averaged ± 5 per cent. Recoveries of added 3-methoxy-4-hydroxymandelic acid, 0.5 to 6 μg, were 95 ± 4 per cent.

Preformed urinary creatinine was determined by the method of van Pilsum, Martin, Kito and Hess (15).

RESULTS

Table I shows a comparison between the average urinary adrenaline output before and after centrifugation and before and after a mock centrifugation. From Table I, it is apparent that there is a 60 per cent increase in adrenaline output before actual centrifugation, and 51 per cent increase is seen before a mock centrifugation. There is a significant increase in adrenaline output after centrifugation as well as after a mock ride (Figure 1). It should be emphasized that the adrenaline output following centrifugation is not significantly different from that following mock centrifugation.

Table II shows a comparison between the urinary output of noradrenaline before and after centrifugation and before and after a mock centrifugation. From this table it is apparent that there is no significant increase in the noradrenaline output

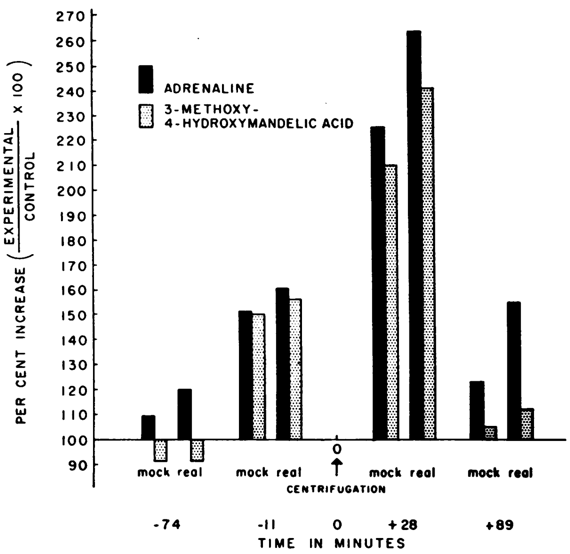

FIG. 1. AVERAGE PER CENT CHANGE OF ADRENALINE BEFORE AND AFTER CENTRIFUGATION AS COMPARED WITH THE PER CENT CHANGE IN 3-METHOXY-4-HYDROXYMANDELIC ACID. All figures reported in micrograms per hour.

immediately prior to both the true and the mock centrifugations. However, it should be noted that the greatest urinary output of noradrenaline follows actual centrifugation and is 53 per cent ($p < 0.05$) greater than the output following the mock centrifugation (Figure 2). Mock centrifugation per se produced an insignificant elevation in the output of noradrenaline.

Table III shows the average hourly rate of urinary excretion of 3-methoxy-4-hydroxymandelic acid before and after centrifugation and before and after mock centrifugation. The figures are the mean values for all subjects at the time of urine collections. From Table III it is evident that there was no significant difference in urinary excretion of 3-methoxy-4-hydroxymandelic acid in a real or mock acceleration. Further, there was a marked increase in the excretion of 3-methoxy-4-hydroxymandelic acid following actual centrifugation as well as following a mock centrifugation. This elevated 3-methoxy-4-hydroxymandelic acid output returned to control levels within 90 minutes after centrifugation.

As is shown in Figures 1 and 2, which illustrate the mean excretion rate of 3-methoxy-4-hydroxymandelic acid in each sampling period as a percentage change from control level, the increase was 56 per cent before a 12 G acceleration and 50 per cent prior to a mock centrifuge ride. In the period encompassing a real acceleration, there was an average increase of 141 per cent ($p < 0.001$). For mock accelerations, during this period, the increase was 110 per cent ($p < 0.01$).

Changes in creatinine excretion rate before and after real and mock acceleration were not statistically significant. Since the creatinine content of the urine has been used as the basis of determining the amount of 3-methoxy-4-hydroxymandelic acid present, the micrograms of 3-methoxy-4-hydroxymandelic acid per milligram of creatinine were calculated for each subject at the time of urine collection (Table III).

Figure 1 is a block graph showing a comparison between the adrenaline output and the 3-methoxy-4-hydroxymandelic acid output during similar periods before and after real and mock centrifugation. From this graph it is quite apparent that as the adrenaline release increases, the 3-methoxy-4-hydroxymandelic acid output also increases.

Figure 2 is a block graph showing a compari-

TABLE II

Average rate of urinary excretion of noradrenaline in response to real and mock centrifugation in micrograms per hour

	Control	Time in minutes				
		−74	−11	0	+28	+89
Real centrifugation	1.56	1.67	2.20	Centrifugation	2.45	2.22
SD	±0.71	±1.17	±0.28		±1.09	±1.45
p*		<0.9	<0.2		<0.05	<0.3
Mock centrifugation	1.56	1.44	1.73		1.61	1.26
SD	±0.71	±0.39	±0.82		±0.69	±0.59
p*		<0.8	<0.8		<0.9	<0.5

* Comparison of experimental with control.

TABLE III

Average rate of urinary excretion of 3-methoxy-4-hydroxymandelic acid in response to real and mock centrifugation

	Control	Time in minutes				
		−74	−11	0	+28	+89
Real centrifugation						
Mean 3-methoxy-4-hydroxymandelic acid excretion (μg/hr)	107.4	101.3	168.6	Centrifugation	259.2	120.9
SD	±30.79	±29.20	±90.66		±97.84	±19.78
p*		<0.7	<0.1		<0.001	<0.8
Mean 3-methoxy-4-hydroxymandelic acid excretion (μg/mg creatinine)	1.5	1.7	2.4		3.2	1.6
Mock centrifugation						
Mean 3-methoxy-4-hydroxymandelic acid excretion (μg/hr)	107.4	102.5	160.7		226.0	112.9
SD	±30.79	±30.83	±105.70		±83.50	±42.60
p*		<0.8	<0.2		<0.01	<0.8
Mean 3-methoxy-4-hydroxymandelic acid excretion (μg/mg creatinine)	1.5	1.7	2.3		3.2	1.6

* Comparison of experimental with control.

son between the noradrenaline output and the 3-methoxy-4-hydroxymandelic acid during similar periods before and after real and mock centrifugation. From Graph 2 it seems evident that, as the noradrenaline output rises, there is a concomitant rise in the 3-methoxy-4-hydroxymandelic acid output. However, this correlation of noradrenaline with 3-methoxy-4-hydroxymandelic acid is not so clearly delineated as it is with adrenaline.

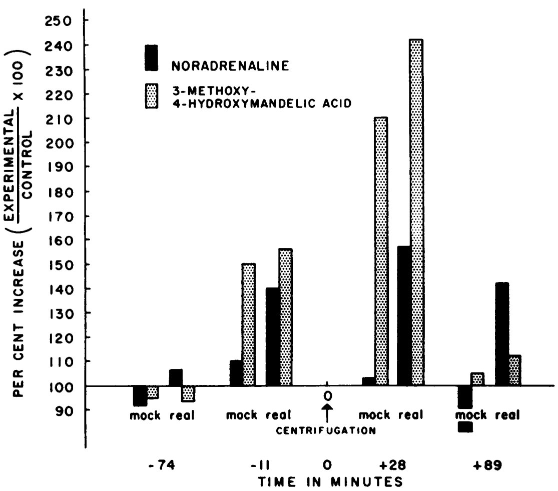

FIG. 2. AVERAGE PER CENT CHANGE OF NORADRENALINE BEFORE AND AFTER CENTRIFUGATION AS COMPARED WITH THE PER CENT CHANGE IN 3-METHOXY-4-HYDROXYMANDELIC ACID. All figures reported in micrograms per hour.

DISCUSSION

Of the adrenaline and noradrenaline normally released by the adrenal medulla and sympathetic nerves, only 3 to 4 per cent is excreted in the urine in the free and conjugated form (8, 9, 16, 17). The remainder of the released adrenaline and noradrenaline is rapidly metabolized and of the metabolic products, 3-methoxy-4-hydroxymandelic acid represents approximately 27 per cent of the metabolized adrenaline and 32 per cent of the metabolized noradrenaline (8, 9). Therefore, in view of 3-methoxy-4-hydroxymandelic acid's common origin, it is quite natural that any stressful situation which would activate the sympathetic nervous system into releasing noradrenaline and the adrenal medulla into releasing adrenaline would also produce an increase in the urinary 3-methoxy-4-hydroxymandelic acid. The experiments herein described illustrate just such a relation; i.e., with an increase in noradrenaline or an increase in adrenaline output, there is a commensurate rise in the 3-methoxy-4-hydroxymandelic acid excretion. Although 3-methoxy-4-hydroxymandelic acid is an excellent indicator of the sympatho-adrenal activity, it does not tell precisely what part of the sympatho-adrenal system is involved. In contrast, urinary adrenaline and noradrenaline do afford more precise information, since adrenaline is derived from the adrenal medulla (18–20) and noradrenaline chiefly from the sympathetic nerves (20–23).

From Table I and Figure 1 it appears that the adrenaline release is closely geared to the emotions. This is evidenced by the markedly increased adrenaline release during the mock ride, that is to say, in anticipation of being centrifuged. Further, the adrenaline output prior to actual centrifugation and prior to a mock ride are similarly elevated; this would indicate that the anxiety associated with being centrifuged or anticipation of being centrifuged, as seen in the mock ride, is the chief factor concerned with the release of adrenaline. Certainly, the physical changes produced by actual centrifugation must cause some release of adrenaline but, at least in these experiments, these physical changes when compared with the emotional effect perform a relatively minor role in the release of adrenaline.

Noradrenaline is the neurohormone of the sympathetic nerves (21, 23, 24) and is of fundamental importance in the control of the vascular bed (24–26). Therefore one would expect that, under the stress of centrifugation, this hormone should be released in increased quantities and in amounts related to the extent of the stress. Table II and Figure 2 show that, following centrifugation, there is a 53 per cent ($p < 0.05$) increase in noradrenaline output. However, in contrast to actual centrifugation, mock acceleration showed little change (3 per cent) in the urinary output of noradrenaline. In general, the pre-centrifugation and pre-mock centrifugation outputs of noradrenaline were not significantly above the control values for noradrenaline (Table II). From these findings and those of other investigators (3), one would conclude that the physical stress of centrifugation activates the sympathetic nervous system and that this is manifested by an increase in the urinary output of noradrenaline. However, it should be pointed out that in these particular experiments, the physical stress of forward acceleration was for only 3 minutes and therefore, minimally activated the sympathetic nervous system, as was reflected by a slight increase in the noradrenaline output. The emotions or anticipation of being centrifuged appear to have little or no effect upon the release of noradrenaline. This is evidenced by the slight but insignifiant increase in noradrenaline release prior to and following a mock centrifugation (Table II and Figure 2).

This elevation of adrenaline associated with the anxiety of anticipating centrifugation and the elevation of noradrenaline following actual centrifugation emphasize the possible importance of these two hormones in various mental and physical stresses. Furthermore, the previous report of Goodall (19) that the adrenals of "fright and flight" animals, as do those of man (18, 24) contain predominantly adrenaline, becomes more meaningful when extrapolated in terms of anxiety and the preparation for flight; the adrenals of aggressive animals such as the lion contain predominantly noradrenaline. Adrenaline elevates the metabolism (24, 27, 28), increases the cardiac rate (29–31) and is a vasodilator (24, 32) in the muscles commonly involved in running; noradrenaline, on the other hand, is a potent vasoconstrictor (24, 32) with limited metabolic action (24, 28, 33, 34).

SUMMARY

The purpose of these experiments was to determine the effect of centrifugation (gravitational stress) and the anticipation of being centrifuged upon the release of adrenaline, noradrenaline, and their common metabolic product, 3-methoxy-4-hydroxymandelic acid. Nine Air Force subjects were centrifuged at 1 G per 5 seconds to 12 G or were given a mock ride. Each subject was unaware as to whether he would receive a real ride or a mock ride. It was therefore possible to measure the sympatho-adrenal response to both centrifugation and anticipation of centrifugation. Urine samples were collected before and after each ride and bioassayed for adrenaline and noradrenaline; 3-methoxy-4-hydroxymandelic acid was determined chromatographically.

Under high gravitational stress, increased adrenaline release seems to be largely related to the emotions, while the noradrenaline release seems more closely related to the physical changes (hemodynamics and so forth) produced by centrifugation. Following the increased release of either or both adrenaline and noradrenaline, there is a commensurate rise in the urinary output of their common metabolic product, 3-methoxy-4-hydroxymandelic acid.

REFERENCES

1. Goodall, McC., Stone, C., and Haynes, B. W., Jr. Urinary output of adrenaline and noradrenaline in severe thermal burns. Ann. Surg. 1957, **145**, 479.
2. von Euler, U. S., and Hellner, S. Excretion of noradrenaline and adrenaline in muscular work. Acta physiol. scand. 1952, **26**, 183.
3. Goodall, McC., and Meehan, J. P. Correlation of 'g' tolerance to urinary adrenaline and noradrenaline. Proc. Amer. Physiol. Soc. Meeting, Rochester, 1956.
4. Zuidema, G. P., Silverman, A. J., Cohen, S. I., and Goodall, McC. Catechol amine and psychologic correlates of vascular responses. New Engl. J. Med. 1957, **256**, 976.
5. Berman, M. L. Quantitative estimation of a urinary metabolite of adrenaline and noradrenaline as possible indication of tolerance to gravitational stress. Aerospace Med. 1959, **30**, 580.
6. Franksson, C., Gemzell, C. A., and von Euler, U. S. Cortical and medullary adrenal activity in surgical and allied conditions. J. clin. Endocr. 1954, **14**, 608.
7. Armstrong, M. D., McMillan, A., and Shaw, K. N. 3-Methoxy-4-hydroxy-D-mandelic acid, a urinary metabolite of norepinephrine. Biochim. biophys. Acta 1957, **25**, 422.
8. Goodall, McC., Kirshner, N., and Rosen, L. Metabolism of noradrenaline in the human. J. clin. Invest., 1959, **38**, 707.
9. Goodall, McC. Metabolic products of adrenaline and noradrenaline in human urine. Pharmacol. Rev. 1959, **11**, 416.
10. LaBrosse, E. A., Axelrod, J., and Kety, S. S. O-Methylation, the principal route of metabolism of epinephrine in man. Science 1958, **128**, 593.
11. Clarke, N. P., Hyde, A. S., Cherniack, N. S., and Lindberg, E. F. A preliminary report of human response to rearward facing re-entry accelerations. Wright Air Development Center Tech. Note. 1959, 59–109.
12. von Euler, U. S., and Hellner, S. Excretion of noradrenaline, adrenaline, and hydroxytyramine in urine. Acta physiol. scand. 1951, **22**, 161.
13. Armstrong, M. D. Estimation of 3-methoxy-4-hydroxymandelic acid for the detection of pheochromocytoma. Privately circulated by the author, Fels Research Institute, Yellow Springs, Ohio, 8 pp.
14. Berman, M. L., and Pettitt, J. Urinary excretion of 3-methoxy-4-hydroxymandelic acid following several stress situations. J. Lab. clin. Med. Dec., 1960. In press.
15. van Pilsum, J. F., Martin, R. P., Kito, E., and Hess, J. Determination of creatine, creatinine, arginine, guanidoacetic acid, guanidine, and methylguanidine in biological fluids. J. biol. Chem. 1956, **222**, 225.
16. von Euler, U. S., Luft, R., and Sundin, T. Excretion of urinary adrenaline in normals following intravenous infusion. Acta physiol. scand. 1954, **30**, 249.
17. von Euler, U. S., and Luft, R. Noradrenaline output in urine after infusion in man. Brit. J. Pharmacol. 1951, **6**, 286.
18. von Euler, U. S., Franksson, C., and Hellstrom, J. Adrenaline and noradrenaline content of surgically removed human suprarenal glands. Acta physiol. scand. 1954, **31**, 6.
19. Goodall, McC. Studies of adrenaline and noradrenaline in mammalian heart and suprarenals. Acta physiol. scand. 1951, **24**, suppl. 85.
20. von Euler, U. S., Franksson, C., and Hellstrom, J. Adrenaline and noradrenaline output in urine after unilateral and bilateral adrenalectomy in man. Acta physiol. scand. 1954, **31**, 1.
21. von Euler, U. S. Identification of the sympathomimetic ergone in adrenergic nerves of cattle (sympathin N) with laevo-noradrenaline. Acta physiol. scand. 1948, **16**, 63.
22. von Euler, U. S. The nature of adrenergic nerve mediators. Pharmacol. Rev. 1951, **3**, 247.
23. Goodall, McC., and Kirshner, N. Biosynthesis of epinephrine and norepinephrine by sympathetic nerves and ganglia. Circulation 1958, **17**, 366.
24. von Euler, U. S. Noradrenaline. Springfield, Ill., Charles C Thomas, 1956.
25. Burn, J. H. Functions of autonomic transmitters. Baltimore, Williams and Wilkins, 1956.
26. Kappert, A., Sutton, G. C., Reale, A., Skoglund, K. H., and Nylin, G. The clinical response of human beings to l-nor-adrenaline and its clinical applicability. Acta cardiol. (Brux.) 1950, **5**, 121.
27. Garcia-Ramos, J., and de Arellano, J. R. Los efectos de la adrenalina sobre el consumo de oxígeno del corazón. Arch. Inst. Cardiol. Méx. 1951, **21**, 205.
28. Graham, J. D. P., and James, D. M. The glycaemic response of rabbits to L-adrenaline and L-noradrenaline and the effect thereon of dimercaprol. J. Physiol., 1952, **118**, 479.
29. Barcroft, H., and Konzett, H. Action of noradrenaline and adrenaline on human heart-rate. Lancet 1949, **1**, 147.
30. Gollwitzer-Meier, K., Kramer, K., and Kruger, E. Die Wirkung des Adrenalis auf die Energetik des Herzens. Pflüg. Arch. ges. Physiol. 1936, **237**, 639.
31. Nathanson, M. H., and Miller, H. The action of nor-epinephrine and of epinephrine on the ventricular rate of heart block. Amer. Heart J. 1950, **40**, 374.
32. Goldenberg, M., Pines, K. L., Baldwin, E. de F., Greene, D. G., and Roh, C. E. The hemodynamic response of man to nor-epinephrine and epinephrine and its relation to the problem of hypertension. Amer. J. Med. 1948, **5**, 792.
33. Reale, A., Kappert, A., Skoglund, C.-H., and Sutton, G. C. The effect of L-Nor-Adrenaline on the oxygen consumption of human beings. Acta physiol. scand. 1950, **20**, 153.
34. Lundholm, L. The effect of *l*-noradrenalin and ergotamine on the oxygen consumption of guinea-pigs. Acta physiol. scand. 1949, **18**, 341.