4. The procedure provides a rapid method for the determination of 17-oxo steroid sulphate/glucuronide ratios and forms the basis of a method for the partial purification of steroid glucuronides.

5. Attempts to isolate individual conjugates by gradient elution on these columns have not been successful.

REFERENCES

- Abul-Fadl, M. A. M. (1957). *Biochem. J.* **65**, 16p.
 Allen, W. M. (1950). *J. clin. Endocrin. Metab.* **10**, 71.
 Ayres, P. J., Barlow, J. J., Garrod, O., Kellie, A. E., Tait, S. A. S., Tait, J. F. & Walker, G. (1957). *An International Symposium on Aldosterone*, p. 73. Ed. by Muller, A. F. & O'Connor, C. M. London: Churchill.
 Barlow, J. J. (1957). *Biochem. J.* **65**, 34p.
 Brooks, C. J. W. & Norymberski, J. K. (1953). *Biochem. J.* **55**, 371.
 Callow, N. H., Callow, R. K. & Emmens, C. W. (1938). *Biochem. J.* **32**, 1312.
 Dodgson, K. S. & Spencer, B. (1953). *Biochem. J.* **55**, 315.
 Dreker, I. J., Heisler, A., Scism, G. R., Stern, S., Pearson, S. & McGavack, T. H. (1952). *J. clin. Endocrin. Metab.* **12**, 55.
 Edwards, R. W. H. & Kellie, A. E. (1958). *Acta endocr., Copenhagen*, **27**, 262.
 Edwards, R. W. H., Kellie, A. E. & Wade, A. P. (1953). *Mem. Soc. Endocrin.* **2**, 53. London: Dobson.
 Kellie, A. E. & Wade, A. P. (1956). *Acta endocr., Copenhagen*, **23**, 357.
 Lieberman, S. & Dobriner, K. (1948). *Recent Progr. Hormone Res.* **3**, 71.
 Mead, J. A. R., Smith, J. N. & Williams, R. T. (1958). *Biochem. J.* **68**, 61.
 Norymberski, J. K. (1952). *Nature, Lond.*, **170**, 1074.
 Paul, J. (1951). Ph.D. Thesis: University of Glasgow.
 Reichstein, T. & Shoppee, C. W. (1949). *Disc. Faraday Soc.* **7**, 305.
 West, C. D., Reich, H. & Samuels, L. T. (1951). *J. biol. Chem.* **193**, 219.
 Wotiz, H. H., Lemon, H. M., Marcus, P. & Savard, K. (1957). *J. clin. Endocrin. Metab.* **17**, 534.

Seasonal Changes of the Catechol Compounds Present in Rat Tissues

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Raab (1941*a*, *b*, 1943*a*), using Shaw's colorimetric method of estimation, reported that the concentrations of 'adrenaline-like catechols' in rat tissues were higher in winter than in summer. This has been confirmed in the present experiments with the ethylenediamine method of Weil-Malherbe & Bone (1953); these experiments have also shown that lower amounts of catechol compounds are present in spring and early autumn (Montagu, 1956*a*).

The fluorescence produced in the ethylenediamine reaction was previously attributed to noradrenaline and adrenaline (Montagu, 1956*b*). Further investigation has shown that a third basic compound is sometimes present (Montagu, 1957). Catechol compounds other than noradrenaline and adrenaline have been found in several animal tissues: examples are 3-hydroxytyramine in sheep heart (Goodall, 1950), in adrenals of sheep and cattle (Shepherd & West, 1953; Holtz & Westermann, 1956) and in phaeochromocytoma (Weil-Malherbe, 1956); *isopropyl*noradrenaline in cat adrenals (Lockett, 1954); 3:4-dihydroxyphenylalanine in ox adrenals (Werle & Jüntgen-Sell, 1955) in phaeochromocytoma (Weil-Malherbe, 1956) and

in a human brain (Montagu, 1957). 3:4-Dihydroxyphenylacetic acid is present in urine (Euler, Euler & Floding, 1955; Weil-Malherbe & Bone, 1957).

METHODS

The experiments date from April, 1954. Male rats of the Wistar strain, isolated from females, were used. They received a standard diet (diet 41; Bruce & Parkes, 1949, 1950) and drinking water *ad lib*. They were housed in a separate building 16 yards from the Laboratory, exposed to daylight and kept, from October until May, at 65–70° F. They were killed in the morning by a blow on the head within 15 min. of being brought in from the animal house.

Estimation of 'total catechols'. The heart, brain, spleen, diaphragm muscle, both kidneys and part of the liver and of the leg muscles of a single rat were separately extracted and treated as already described (Montagu, 1956*b*). The fluorescence in the final solution was measured with yellow and blue-green filters after condensation with ethylenediamine dihydrochloride (ED). As catechol compounds other than adrenaline and noradrenaline may be present, the calculation previously used may not be valid. The fluorescence produced by the mixture of catechol compounds was therefore compared with that of a standard solution of adrenaline, and the concentration of 'total catechols' calculated in terms of this compound.

During the 3½ years' period of this investigation three modifications in the method were introduced: (1) in April

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1955 the fluorimeter was modified as described by Weil-Malherbe & Bone (1957); (2) in November 1955 the phosphate solution used for the partial neutralization of the crude tissue extracts was replaced by 1 ml. of 1% (w/v) ascorbic acid and 5 ml. of a solution containing 2% (w/v) of $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ and 1% of disodium ethylenediaminetetraacetate (pH 7.2); (3) from May 1956 estimation of the separate catecholamines was also carried out and larger amounts of tissue were homogenized in 4 vol. of acid methanol and re-extracted once instead of twice with a further 2 vol. For liver, the extracts were acidified to pH 3 with 5N-HCl, and half or more of the methanol was then removed by distillation *in vacuo* at 37° to reduce the amounts of *n*-butanol and HCl required in the subsequent extraction. After the tissue extracts had been filtered through alumina, 'total catechols' were estimated in a sample of the eluate and the rest was analysed further as described below.

Estimation of catecholamines. Basic compounds were separated from neutral and acidic components by treatment with the cation-exchange resin Amberlite IRC-50 (British Drug Houses Ltd., analytical grade, 16–50 mesh) buffered at pH 6.5, the procedure following approximately that of Bergström & Hansson (1951). The moist resin (2 g.) in a column of 6 mm. bore was prepared for use by treating it with 25 ml. quantities of N-HCl, water, N-NaOH, water, 0.5M-phosphate buffer (pH 6.5) and water at a filtration rate not exceeding 1 ml./min. Recycling was continued until the HCl filtrate gave no fluorescence on treatment with ED. The tissue extract was then mixed with 0.1 vol. of 1% disodium ethylenediaminetetraacetate, and adjusted to pH 6.5 ± 0.1 and filtered through Whatman no. 5 paper to remove traces of alumina; it was poured on to the column of resin, the column was washed with 25 ml. of 0.2% NaCl and the alkaline compounds were eluted with 30 ml. of N-HCl at a rate not exceeding 0.8 ml./min. After recycling once, the column was ready for use again. A part of the eluate, magnetically stirred, was adjusted to pH 3.5 by dropwise addition of N-NaOH, and was used for estimation of the total basic fraction by the ED method, and of noradrenaline and adrenaline by the trihydroxyindole (THI) method of Euler & Floding (1955). In this last method all solutions, including standards of adrenaline and noradrenaline, were diluted to 5 ml. with approx. 0.3 MN-HCl before estimation. The rest of the procedure was as described by Weil-Malherbe & Bone (1957) except that the alkaline ascorbate contained only 0.72 ml. of 2% (w/v) ascorbic acid and the final volumes were not made up to 10 ml.

The estimates of noradrenaline and adrenaline were often insufficient to account for the fluorescence produced in the ED method. The concentration of a third compound, X, which is probably hydroxytyramine (Montagu, 1957), was calculated in terms of this compound as follows: let a , n and h denote the ED fluorescence (yellow filter) produced by 200 μmg . of pure adrenaline, noradrenaline and hydroxytyramine respectively, and let x denote the ED fluorescence produced by the eluate sample. If A and N are the amounts, in μmg ., of adrenaline and noradrenaline found in an identical sample by the THI method, the amount of hydroxytyramine is $(200x - aA - nN)/h \mu\text{mg}$.

Estimates of catecholamines were not corrected for the oxidation of between 4 and 15% of the noradrenaline which occurred at pH 3.5 in the THI method. There was no

seasonal variation in the extent of this oxidation. Correction would have increased noradrenaline and reduced adrenaline estimates by about 9.5% and about 4% respectively of the noradrenaline concentrations shown in the results. The latter factor is relatively large. The effects on X would be negligible. The ascorbic acid added in the THI method produced slight fluorescence, but the reagent blank was not corrected for this.

Statistical analyses. The significance of the estimated amounts of catechol compounds and of their seasonal and other changes has been investigated by Student's *t*-test (McNemar, 1949). Occasionally the estimated amount of basic catechol compounds was less than that of the sum of noradrenaline and adrenaline, leading to a negative estimate of the concentration of X. Such negative estimates are clearly due to experimental error and have been included for statistical analysis.

Operations. Bilateral adrenalectomy or bilateral adrenal demedullation was performed in one stage with ether-air anaesthesia. The rats were maintained on the standard diet with 1% NaCl solution instead of drinking water. Water was restored to demedullated rats 2–3 weeks after operation. Demedullation was accomplished by expressing the contents of the adrenal capsule; the cortex regenerates within a month (Ingle & Griffith, 1949), as was shown by a normal insulin tolerance and by histological examination of representative sections from eight glands. A trace of chromaffin tissue was found in one. The insulin tolerance of rats adrenalectomized 2–3 weeks previously was about one-hundredth of that of normal rats. Rats which had received insulin were not used in the experiments reported below.

RESULTS

3-Hydroxytyramine, isopropylnoradrenaline, 3:4-dihydroxyphenylalanine (DOPA) and 3:4-dihydroxyphenylacetic acid (DOPAC) are all adsorbed by alumina at pH 8.4 and form fluorescent compounds in the ED reaction. The fluorescence intensities of these compounds relative to that of the adrenaline derivative are shown in Table 1. In the isobutanol phase the ratio of the fluorescence measured with the blue-green filter to that with the yellow filter is unity with adrenaline and isopropylnoradrenaline, but is greater with each of the other compounds. With DOPA and DOPAC, unlike the basic compounds, the fluorescence in the isobutanol phase is less than that in the aqueous phase.

The concentrations of 'total catechols' in tissues have been measured with yellow and blue-green filters and are expressed accordingly. The ratio of the blue-green to the yellow fluorescence was not constant, showing that the proportion of the various components varies, and that the calculated amounts expressed in terms of adrenaline have only qualitative significance. The amounts of total catechols varied significantly from season to season whether comparison was made between all the results obtained in each month during the whole period of study, or whether the results of each year were considered separately. Concentrations in each

Table 1. *Fluorescence intensities of equimolecular amounts of various catechol compounds after condensation with ethylenediamine*

Intensities are expressed relative to those of adrenaline, arbitrarily taken as 100 with each filter in the isobutanol phase. Examples of the fluorescence in extracts of equal weights of rat heart and brain, eluted from alumina and condensed with ED, are included.

Compound or tissue	isoButanol phase		Ratio (B/Y)	Aqueous phase with yellow filter
	With yellow filter (Y)	With blue-green filter (B)		
Adrenaline	100	100	1.0	2
Noradrenaline	43	145	3.4	2
3-Hydroxytyramine	38	67	1.8	2
isoPropylnoradrenaline	52	52	1.0	2
3:4-Dihydroxyphenylalanine	0.9	1.8	2.0	5
3:4-Dihydroxyphenylacetic acid	6.8	25	3.7	11
Rat heart	40	122	3.1	3
Rat brain	62	149	2.4	4

Table 2. *Concentrations of 'total catechols' in heart estimated by the ethylenediamine method with the yellow filter*

Results are expressed as $\mu\text{mg./g.}$ of heart (wet wt.) $\pm$ s.e. Black figures denote the numbers of estimations.

	April	May	June	July	August	September
April 1954– March 1955	41.8 1	239 $\pm$ 46.3 4	191 $\pm$ 22.3 4	443 $\pm$ 66.7 7	262 $\pm$ 78.0 5	264 $\pm$ 61.6 6
April 1955– March 1956	— 1	346 1	—	—	—	—
April 1956– March 1957	— 1	168 1	206 $\pm$ 46.6 4	234 $\pm$ 12.0 2	228 1	243 $\pm$ 35.0 2
April 1957– August 1957	211 $\pm$ 30.3 5	283 $\pm$ 16.5 3	324 $\pm$ 14.6 2	—	173 $\pm$ 52.0 2	—
	October	November	December	January	February	March
April 1954– March 1955	396 $\pm$ 46.9 11	451 $\pm$ 147 4	502 $\pm$ 35.7 13	—	398 $\pm$ 2.0 2	169 $\pm$ 14.5 2
April 1955– March 1956	— —	603 $\pm$ 269 7	1690 1	691 $\pm$ 164 4	—	220 $\pm$ 80.9 5
April 1956– March 1957	334 1	446 $\pm$ 41.3 3	535 $\pm$ 175 2	319 $\pm$ 21.1 3	—	212 $\pm$ 15.7 3
April 1957– August 1957	—	—	—	—	—	—

The significance (*P*) of differences of concentration in several groups of months, denoted by Roman numerals. The low concentrations found in spring and autumn were combined and tested for significance against the higher concentrations in summer and in winter.

	Summer	Winter
April 1954– March 1955	(IV + VIII + IX) 1954 + (III) 1955 VII; <i>P</i> = 0.02	XI + XII + I; <i>P</i> < 0.001
April 1955– March 1956	(V) 1955 + (III + V + VI) 1956 (No estimations)	XI + XII + I; <i>P</i> < 0.02
April 1956– March 1957	(IV + VIII + IX) 1956 + (III) 1957 VII, or V to VII, not significant	XI + XII + I; <i>P</i> < 0.01
April 1957– August 1957	(III + IV + VIII) 1957 V to VII; <i>P</i> < 0.01	(No estimations)
April 1954– August 1957	(III + IV + VIII + IX) VII; <i>P</i> < 0.01	XI + XII + I; <i>P</i> < 0.001

month are shown in Tables 2 and 3. They were lowest in March to April and in August and September, and were highest in the winter (December, January or February). Increases also occurred during the summer (May to July), but were smaller except in liver. The changes of concentration in the seven tissues have been combined in Fig. 1; this shows that the ratio of the fluorescence with the blue-green filter to that with the yellow was unusually low in February and July.

Compounds producing the ethylenediamine fluorescence in tissue extracts. Catecholamines may be separated from acidic and neutral compounds by adsorption of the catecholamines on Amberlite IRC-50 resin at pH 6.5. On elution with acid the ED fluorescence of the eluate was 85–100 % of its value before treatment with resin, and the ratio of yellow to blue-green fluorescence was unchanged. Pure solutions of adrenaline and noradrenaline gave recoveries of 90–100 %. Before treatment with the resin the fluorescence in the aqueous phase was variable but small, and was not significantly changed after the removal of any non-basic com-

pounds, such as DOPA or DOPAC, which might have been present. There seems to be no evidence for the presence of these compounds in the tissue extracts. DOPA forms a fluorescent derivative in the THI method (Weil-Malherbe & Bone, 1957), and the fluorescence is greater after oxidation at pH 6.0 than at pH 3.5. The filtrates from resin columns were equally fluorescent after oxidation at the lower pH.

Catecholamines. The amounts of noradrenaline and adrenaline estimated by the THI method in the acid eluates from the resin were usually insufficient to account for the estimates of the total basic fraction; this is shown in Tables 4 and 5, in which the difference, compound X, has been expressed in terms of hydroxytyramine. Table 5 shows the concentrations in heart and brain in different months; it suggests that there are probably seasonal changes of the concentrations of each of the three catecholamines and of their combined amounts.

The concentration of X was highest in brain and must account for much of the adrenaline previously reported in that tissue (Montagu, 1956*b*). It was

Table 3. 'Total catechols' in various rat tissues estimated in different months by the ethylenediamine method

The yellow filter was used and the results are expressed in $\mu\text{mg.}$ of adrenaline/g. wet wt. of tissue, except for spleen ($\mu\text{mg.}$ of adrenaline/organ). Figures in parentheses indicate the corresponding results obtained with the blue-green filter. Black numbers denote the numbers of estimations.

	April	May	June	July	August	September
Brain	319 (609) 5	411 (714) 1	370 (923) 4	252 (449) 2	290 (726) 2	— 0
Heart	183 (479) 6	258 (722) 9	224 (707) 10	397 (764) 9	236 (545) 8	258 (569) 10
Spleen	125 (375) 7	— 0	151 (556) 4	122 (302) 2	166 (292) 2	137 (281) 2
Diaphragm	85.3 (228) 4	88.0 (126) 1	62.7 (279) 5	153 (357) 7	74.7 (175) 3	122 (284) 7
Kidneys	29.6 (66.6) 4	24.4 (56.4) 1	54.3 (117) 3	104 (189) 4	82.7 (207) 6	60.4 (111) 3
Liver	21.3 (58.3) 15	49.4 (145) 6	47.6 (108) 8	70.6 (124) 10	41.9 (112) 3	46.5 (106) 8
Leg muscles	13.6 (44.9) 2	31.2 (65.7) 3	17.6 (34.5) 3	48.1 (102) 4	16.0 (35.1) 2	23.0 (47.4) 7
	October	November	December	January	February	March
Brain	— 0	480 (1040) 9	532 (1120) 4	640 (1350) 4	584 (982) 2	391 (731) 10
Heart	390 (971) 12	534 (1340) 13	580 (1510) 17	531 (1670) 7	398 (1020) 2	208 (498) 10
Spleen	— 0	144 (435) 7	192 (671) 3	327 (1280) 5	— 0	114 (234) 6
Diaphragm	— 0	141 (390) 6	288 (1020) 4	198 (741) 12	219 (252) 1	67.9 (209) 6
Kidneys	113 (296) 10	148 (434) 6	156 (455) 18	144 (468) 5	153 (303) 2	60.0 (129) 7
Liver	— 0	51.8 (137) 17	45.0 (113) 5	65.7 (190) 12	79.9 (185) 3	24.1 (64.4) 11
Leg muscles	— 0	33.2 (77.3) 4	33.2 (81.2) 1	43.2 (115) 7	44.9 (90.8) 1	16.5 (77.9) 1

Fig. 1. Seasonal changes of the concentrations of total catechols, estimated by the ethylenediamine method with yellow (Δ) and blue-green ($\circ$) filters, in rat heart, brain, spleen, kidney, diaphragm, liver and leg muscles. Considering each tissue separately, the concentrations for each month in the different years were calculated as percentages of those in January of the following year, and were denoted by m . A loaded mean for all tissues in each month was then calculated from the formula

$$\bar{m} = \frac{\sum(\rho m)}{\sum \rho},$$

where ρ was either the number of estimates in January, or the number of estimates in the particular month in question, whichever was the smaller.

also found in liver, but only sometimes in spleen, heart, kidneys and diaphragm. The mean concentrations found in heart in each of the months March, April, June, August and September, and the mean concentration in diaphragms, were not statistically significant.

Comparison of normal, adrenalectomized and demedullated rats. Some experiments were carried out to investigate the possible dependence of the seasonal changes on the activity of the adrenal medulla or cortex. Rats were bilaterally adrenalectomized in September–October, when concentrations of ‘total catechols’ are increasing, and in January–February when they are decreasing; they were killed some weeks later at the same time as control rats and the hearts were analysed. It was found that when adrenalectomy had been performed in the autumn the amounts of total catechols 6–10 weeks later were lower in the adrenalectomized rats than in their controls, but that the reverse was the case when the operation was performed in January–February (Table 6, *a-b*). These two effects of adrenalectomy were statistically different whether the results were expressed as concentrations or as $\mu\text{mg./whole heart}$. The results in Table 6 have been expressed as $\mu\text{mg./organ}$, as

heart weights were significantly higher ($P < 0.01$) in the adrenalectomized rats; the weights of these rats were similar to those of their controls at operation and *post mortem*. Table 6 also shows the changes which were found in the amounts of the individual catecholamines. The table suggests that seasonal changes of catechol compounds in heart are reduced by adrenalectomy.

Rats adrenalectomized only 11 days previously were compared with the control rats used in some of the above experiments (Table 6, *c-d*). Here also the effects of operation depended on the time of year at which it was carried out, and the changes of total catechols produced by adrenalectomy in November were probably significantly different from those produced in March. They were smaller than in rats adrenalectomized less recently and differences of heart weight were not detected. In five experiments cortisone acetate (3 mg. intraperitoneally for 3 days before killing) had no significant effects on the amounts of total catechols or of catecholamines in hearts of rats adrenalectomized 11 days before analysis in November.

In some of the above experiments brains were also analysed. No significant effects of adrenalectomy were found (Table 6, *e-g*).

In other experiments rats were bilaterally demedullated in September, when concentrations of catechol compounds are low, and were compared with control rats 5–7 weeks later (Table 7). Demedullation had not significantly altered the concentration of total catechols in heart, but had increased ($P < 0.01$) the ratio of the ED fluorescence with blue-green and yellow filters. The total catechols in kidneys of demedullated rats were lower than in the control rats analysed simultaneously, but were probably higher than in intact rats analysed at the time of operation.

DISCUSSION

The concentrations of total catechols, estimated by the ethylenediamine method in seven rat tissues, increase more extensively in the winter than can be accounted for by the minor changes of tissue weight which may occur (see Raab, 1941*a, b*). Non-basic compounds contribute little if at all to the ED estimates of total catechols, and the seasonal changes are therefore changes of basic compounds. The available evidence (see Fig. 1 and Tables 1 and 5) suggests that the concentrations of noradrenaline, adrenaline and of X increase progressively from September to December, with some conversion of X into noradrenaline in January and of noradrenaline into adrenaline in February. Fig. 1 also suggests that a similar sequence may occur between April and July; summer increases are smaller and less easily established.

Experiments were carried out to investigate the possible relation to the adrenal glands of the seasonal changes occurring between autumn and spring. The amounts of total catechols in heart appeared to increase after removal of the adrenals in the autumn, and to decrease when adrenalectomy was performed in January. The changes were probably smaller than the seasonal changes occurring in normal rats and suggest a partial dependence of these changes on the adrenal gland. It is also possible that after adrenalectomy the concentrations of total catechols find an intermediate level which shows no seasonal change. Experiments with rats which had been adrenalectomized only 11 days previously did not eliminate either possibility.

When rats which had been demedullated in September were killed in November, the concentrations of total catechols in hearts were not different from those in control rats analysed simultaneously; this was in spite of the temporary removal of cortex involved in the process of demedullation. Since the autumnal increase of total catechols seems therefore to occur in the absence of

the medulla the ensuing decrease is unlikely to depend on its presence. An alteration in the demedullated rats of the ratio of the ED fluorescence determined with two filters suggests a reduction of the proportion of adrenaline or X, but this was probably an effect of demedullation only. In contrast with those in heart the total catechols in kidneys were reduced by demedullation; this might have been due to the operation itself or to reduction or prevention of seasonal changes. In brain, an organ generally less susceptible to hormonal influence, there was no evidence that seasonal differences involve the adrenal medulla or cortex.

The seasonal changes of the concentrations of catechol compounds in the different tissues might arise from intrinsic variations of any or all of the various internal secretions. Weather and duration of daylight seem to be the other possibilities; owing to increases of catechol compounds in summer as well as winter neither alone is likely to provide adequate explanation. However, the external temperature was lower, and the estimates of total catechols higher, in the winter of 1955-56 than in

Table 4. *Noradrenaline, adrenaline and compound X in rat tissues*

Results are expressed for spleen in $\mu\text{mg./whole spleen}$, and for diaphragm, kidney and liver in $\mu\text{mg./g. wet wt.}$. Compound X is calculated in terms of hydroxytyramine. Results were obtained in June, July, December, January and April and are listed as means $\pm$ S.E.

	Spleen	Diaphragm	Kidney	Liver
Noradrenaline	144 $\pm$ 21.0	86.5 $\pm$ 12.5	72.8 $\pm$ 11.7	28.6 $\pm$ 7.55
Adrenaline	40.3 $\pm$ 6.90	34.0 $\pm$ 5.51	19.9 $\pm$ 3.10	9.07 $\pm$ 4.32
Compound X	110 $\pm$ 33.8	36.5 $\pm$ 20.6	21.2 $\pm$ 6.11	18.9 $\pm$ 6.45
No. of estimates	7	8	10	11

Table 5. *Concentrations of noradrenaline, adrenaline and compound X in rat heart and brain*

Concentrations are expressed as $\mu\text{mg./g. of tissue (wet wt.)}$ and months are denoted by Roman numerals. Compound X is calculated in terms of hydroxytyramine.

	VI	VII	VIII	IX	XI	XII	I	II	III	IV	V
Heart											
Noradrenaline	230	288	203	168	400	—	439	—	270	248	326
Adrenaline	38.7	23.0	57.6	99.3	109	—	79.0	—	109	58.3	70.2
Compound X	52.2	132	65.4	102	232	—	90.0	—	40.5	41.5	84.0
No. of estimations	5	2	1	4	2	0	2	0	3	4	3
Brain											
Noradrenaline	113	143	132	—	120	190	174	260	198	137	250
Adrenaline	20.9	30.0	15.2	—	75.6	93.2	39.6	72.6	53.0	29.9	35.2
Compound X	427	327	378	—	420	439	835	642	408	332	353
No. of estimations	4	2	2	0	2	3	1	2	4	2	1

Estimates of the significance (P) of differences of concentration in groups of several different months

Heart

Noradrenaline	XI to I > III + IV + VI + VIII + IX	$P < 0.01$
Adrenaline	IX to III > IV to VIII	$P = 0.001$
Compound X	XI to I > III + IV + VI + VIII + IX	$P < 0.01$

Brain

Noradrenaline	XII to III > IV + (VI to VIII) + XI	$P < 0.001$
Adrenaline	XI to II > IV to VIII	$P < 0.001$
Compound X	XII to II > IV + V + VII + VIII	$P = 0.05$

Table 6. *Effects of adrenalectomy, obtained from simultaneous analysis of adrenalectomized and control rats, and the changes occurring after operation as judged from comparison with intact rats analysed at the times of operation*

Pooled rats were used in each experiment. Changes and percentage changes of the amounts, in $\mu\text{mg./whole organ}$, of total catechols, estimated by the ethylenediamine method with yellow and blue-green filters (ED_Y and ED_B), and of noradrenaline (N), adrenaline (A) and of compound X, are shown. The significance P of the effects of adrenalectomy was assessed from t tests of the differences, expressed as percentages, between adrenalectomized and control rats, except for compound X where P was calculated from actual differences. Numbers of experiments listed under 'changes since operation' refer to numbers of analyses made at the times of operation. Time of operation, calculated as a mean date, and of subsequent analysis, is included.

	Effect of adrenalectomy					Change since adrenalectomy			Amounts in adrenalectomized rats ($\mu\text{mg./organ}$)					Rat wt. (g.)		Tissue wt. (g.)
	ED _Y	ED _B	N	A	X	ED _Y	ED _B	ED _Y	ED _B	N	A	X	At operation	Post mortem		
Heart																
(a) Date of operation 3. x. 56; date of analysis 16. xi. 56																
Change	-99.7	-217	+12.5	-36.4				-78.5	+61.0	+180				C.* 154	225	0.658
% change	-30	-26	+1.0	-53				—	+37	+40				O.* 163	219	0.723
P	<0.01	<0.05	>0.2	<0.05				>0.2								
No. of expts.	3	3	2	2				2	3	3	2	2	2			
(b) Date of operation 30. i. 57; date of analysis 27. iii. 57																
Change	+45.0	+128	+44.3	-10.9				+125	-16.0	-149				C. 159	277	0.741
% change	+25	+29	+31	-12				—	-7.0	-0.20				O. 157	276	0.910
P	<0.05	<0.05	>0.2	<0.1				<0.1								
No. of expts.	5	5	4	4				4	3	3	4	4	4			
(c) Date of operation 17. xi. 56; date of analysis 29. xi. 56																
Change	-35.6	-106	-42.6	+5.8				-73.5	—	—				C. 170	180	0.576
% change	-17	-19	-23	+11				—	—	—				O. 168	182	0.587
P	<0.02	<0.02	<0.02	>0.2				<0.2	—	—						
No. of expts.	5	5	5	5				5	—	—						
(d) Date of operation 15. iii. 57; date of analysis 26. iii. 57																
Change	-10.3	-33.3	+12.5	-15.5				+36.7	—	—				C. 237	261	0.777
% change	-6.0	-5.2	+29	-18				—	—	—				O. 240	249	0.716
P	<0.05	>0.2	>0.2	=0.05				<0.1	—	—						
No. of expts.	3	3	3	3				3	—	—						
Brain																
(e) Date of operation 18. x. 56; date of analysis 11. xii. 56																
Change	+1.0	-60.0	+46.0	-62.0				+348	—	—				C. 142	245	1.51
% change	+0.1	-3.5	+18	-31				—	—	—				O. 139	238	1.49
No. of expts.	1	1	1	1				1	1	1	1	1	1			
(f) Date of operation 30. i. 57; date of analysis 27. iii. 57																
Change	+3.2	+44.2	-11.8	+4.2				+107	-287	-428				C. 178	275	1.48
% change	+0.4	+2.7	-2.6	+11				—	-37	-28				O. 168	264	1.53
P	>0.2	>0.2	>0.2	>0.2				=0.2								
No. of expts.	5	5	5	5				5	2	2	5	5	5			
(g) Date of operation 15. iii. 57; date of analysis 26. iii. 57																
Change	-7.0	-99.0	—	—				—	—	—				C. 242	261	1.48
% change	-0.9	-11	—	—				—	—	—				O. 240	249	1.57
P	>0.2	<0.1	—	—				—	—	—						
No. of expts.	3	3	—	—				—	—	—						

* C. = control; O. = operated.

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Table 7. *Effects of bilateral adrenal demedullation on the concentrations of total catechols in heart and kidneys, and the changes occurring after operation*

Single rats were compared on separate occasions. Concentrations of total catechols were estimated in $\mu\text{mg./g.}$ of tissue (wet wt.) by the ethylenediamine method with yellow and blue-green filters (ED_Y and ED_B); t tests were carried out on differences between demedullated and intact rats.

	Effect of operation		Change since operation		Concentrations in operated rats		Rat wt. (g.)		Tissue wt. (g.)
	ED_Y	ED_B	ED_Y	ED_B	ED_Y	ED_B	At operation	Post mortem	
Date of operation 18. ix. 54; date of analysis 24. x. 54									
Heart									
Change	+34.0	+165	+114	+461	437	1140	C.* 205	274	0.811
% change	+8.4	+17	+35	+68	—	—	O.* 194	257	0.760
P	>0.2	<0.2	<0.05	<0.01	—	—			
No. of expts.	10	10	7	7					
Date of operation 18. ix. 54; date of analysis 24. x. 54									
Kidney									
Change	-26.0	-74.0	+29.2	+113	91.0	228	C. 205	274	1.92
% change	-27	-25	+47	+98	—	—	O. 194	257	1.87
P	<0.01	<0.01	<0.05	<0.05	—	—			
No. of expts.	10	10	3	3					

* C. = control; O. = operated.

the preceding or following years. The rats were out-of-doors for less than half a minute shortly before being killed. If the large increases of catechol compounds in heart, which are apparently independent of the adrenal medulla but dependent on the cortex, are due to a brief lowering of temperature, they must be brought about by a mechanism such as, for example, presumably exists for the rapid synthesis or release of catecholamines from adrenergic neurones. It is possible that the generalized sympathetic discharges which result from stimulation of the heat-conserving centre in the hypothalamus (Keller, 1938) depend for their elicitation or effect on the adrenal cortex; it is known that the cortex responds to a reduction of temperature in the absence of circulating adrenaline (Vogt, 1952). Such an explanation would not be enough: the mean temperature in March (42°F.), still well below that in the animal house, is similar to that of November (Manley, 1952); in March the total catechols are at their lowest levels and would probably have been higher had the rats been adrenalectomized early in February. Although varying lengths of days might be associated with changes in the activity of the adrenal glands, it is difficult to attribute the biannual changes of catechol compounds to two environmental factors alone.

The variations of the concentrations of catechol compounds might explain some seasonal changes of metabolism which have been reported. Among these are changes of respiratory quotient (Cori & Cori, 1926) and heat production (Benedict & Macleod, 1929; Sherwood, 1936), of acid production by the gastric mucosa (Mizell, 1955) and of blood lactate (Swan, 1943), seasonal differences in the response to ouabain (Wollenberger & Karsh, 1951) and barbiturates (de Beer, Hjort & Cook, 1939; Weil-Malherbe, 1955), in the ease of falling asleep (Kleitman, 1939; Renton & Weil-Malherbe, 1956) and in the incidence of angina pectoris (Raab, 1943b). That few of these are noted as biannual may be due to the usual method of comparing only winter with summer levels.

SUMMARY

1. The combined concentrations of the catechol compounds in heart, brain, spleen, kidney, liver, diaphragm and leg muscles of rats have been estimated by the ethylenediamine method for periods of 1–3½ years; there were large and significant seasonal changes in each tissue; concentrations were highest in winter and summer.

2. Besides adrenaline and noradrenaline, a third catecholamine is present in brain and liver, and sometimes in spleen, heart, kidneys and diaphragm; concentrations of all three catecholamines probably vary seasonally.

3. Adrenalectomy reduced or abolished seasonal changes in heart, but not those in brain. Changes in heart were unaffected by removal of the adrenal medulla; concentrations of catechol compounds in kidney were reduced.

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REFERENCES

- Benedict, F. G. & Macleod, G. (1929). *J. Nutr.* **1**, 367.
 Bergström, S. & Hansson, G. (1951). *Acta physiol. scand.* **22**, 87.
 Bruce, H. M. & Parkes, A. S. (1949). *J. Hyg., Camb.*, **47**, 202.
 Bruce, H. M. & Parkes, A. S. (1950). *J. Hyg., Camb.*, **48**, 171.
 Cori, G. T. & Cori, C. F. (1926). *Proc. Soc. exp. Biol., N.Y.*, **24**, 123.
 de Beer, E. J., Hjort, A. M. & Cook, C. A. (1939). *J. Pharmacol.* **65**, 46.
 Euler, Chr. von, Euler, U. S. von & Floding, I. (1955). *Acta physiol. scand.* **33**, suppl. 118, p. 32.
 Euler, U. S. von & Floding, I. (1955). *Acta physiol. scand.* **33**, suppl. 118, p. 45.
 Goodall, M. C. (1950). *Nature, Lond.*, **166**, 738.
 Holtz, P., & Westermann, E. (1956). *Biochem. Z.* **327**, 502.
 Ingle, D. J. & Griffith, J. Q. (1949). In *The Rat in Laboratory Investigation*, 2nd ed., p. 445. Ed. by Farris, E. J. & Griffith, J. Q. Philadelphia: J. B. Lippincott Co.
 Keller, A. D. (1938). *J. Neurophysiol.* **1**, 543.
 Kleitman, N. (1939). *Sleep and Wakefulness as Alternating Phases in the Cycle of Existence*. Chicago, Ill.: University of Chicago Press.
 Lockett, M. F. (1954). *J. Physiol.* **124**, 67 P.
 McNemar, Q. (1949). *Psychological Statistics*, pp. 223-6. New York: John Wiley and Sons Inc.
 Manley, G. (1952). *Climate and the British Scene*, p. 254. London: Collins.
 Mizell, S. (1955). *Amer. J. Physiol.* **180**, 650.
 Montagu, K. A. (1956a). *Nature, Lond.*, **178**, 417.
 Montagu, K. A. (1956b). *Biochem. J.* **63**, 559.
 Montagu, K. A. (1957). *Nature, Lond.*, **180**, 244.
 Raab, W. (1941a). *Endocrinology*, **29**, 126.
 Raab, W. (1941b). *Endocrinology*, **29**, 564.
 Raab, W. (1943a). *Biochem. J.* **37**, 470.
 Raab, W. (1943b). *Exp. Med. Surg.* **1**, 188.
 Renton, G. H. & Weil-Malherbe, H. (1956). *J. Physiol.* **131**, 170.
 Shepherd, D. M. & West, G. B. (1953). *J. Physiol.* **120**, 15.
 Sherwood, T. C. (1936). *J. Nutr.* **12**, 223.
 Swan, M. M. (1943). *Amer. J. Physiol.* **140**, 125.
 Vogt, M. (1952). *Proc. 5th Symp. Colston Res. Soc.*
 Weil-Malherbe, H. (1955). *J. ment. Sci.* **101**, 733.
 Weil-Malherbe, H. (1956). *Lancet*, ii, 282.
 Weil-Malherbe, H. & Bone, A. D. (1953). *Lancet*, i, 974.
 Weil-Malherbe, H. & Bone, A. D. (1957). *J. clin. Path.* **10**, 138.
 Werle, E. & Jüntgen-Sell, J. (1955). *Biochem. Z.* **327**, 259.
 Wollenberger, A. & Karsh, M. L. (1951). *J. Amer. pharm. Ass. (Sci. ed.)*, **40**, 637.

The Relationship of Fermentation to Cell Structure in Yeast

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From a biochemical point of view, the sequence of reactions in fermentation and glycolysis is well understood, and many of the individual enzymes have been purified and extensively characterized. However, from a cytological point of view, the relationship of fermentation to cellular structure is far from clear. Certain experiments suggest that the glycolysis reactions are located in the periphery of the cell (Rothstein, 1954a). For example, in the red blood cell, the uptake of ³²P-labelled phosphate apparently proceeds in part via esterification into glycolytic intermediates in the stroma (Gourley, 1952; Prankerd & Altman, 1954). In muscle, radioautographic studies indicate that ³²P-labelled phosphate is esterified in the periphery of the cell

(Causey & Harris, 1951). In yeast cells, results of detailed studies of sugar uptake have also pointed in the same direction (Rothstein, 1954b).

Studies with certain cell-free preparations of yeast suggest a relationship between glycolytic processes and cellular structure. There are basically two types of yeast preparation: the structureless, soluble fermenting system, and the dried-yeast preparations, in which much of the enzyme activity is associated with insoluble cellular residues. In the soluble fermenting systems (press-juice, Lebedev maceration juice, extracts from frozen and thawed cells, and yeast treated by sonic oscillation) certain characteristic properties are found which were described by Harden (1932). Such preparations show a dependence on orthophosphate and possess a characteristic sequence of production of carbon dioxide. In the absence of phosphate, the course of

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