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Alterations in the activities of ornithine and histidine decarboxylases provoked by testosterone in mice

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Summary

1. The urinary excretion of putrescine has been determined in female mice before and during repeated injections of testosterone.

2. Testosterone administration effected a striking increase in the excretion of free putrescine.

3. Ornithine decarboxylase (L-ornithine carboxy-lyase; E.C. 4.1.1.17) and histidine decarboxylase (L-histidine carboxy-lyase; E.C. 4.1.1.22) activities of mouse kidney and liver were examined. In the kidney, following testosterone administration, ornithine decarboxylase activity was found to be substantially elevated, whereas that of histidine decarboxylase was depressed. In the liver, by contrast, the activity levels of these enzymes were not significantly altered by testosterone treatment.

4. The possibility of a functional interrelation between putrescine and histamine, via the two enzyme activities investigated, is discussed.

Introduction

In a study of various aspects of histamine metabolism in female mice it was noted that testosterone administration greatly reduced urinary histamine excretion, an effect attributable to histidine decarboxylase activity of the kidney being reduced to a small fraction of normal (Henningsson & Rosengren, 1972). In these particular experiments urinary histamine was determined by reaction of the amine with 2,4-dinitrofluorobenzene (DNFB) after purification of the urine on an ion exchange resin. On subjecting the resulting DNB-histamine to thin layer chromatography, an additional yellow DNB-product was disclosed, the chemical nature of which could not be established at that time. In further attempts, to be described in the present study, this compound was identified as putrescine. Further, the effect of testosterone on ornithine decarboxylase activities of liver and kidney and on the urinary excretion of putrescine was investigated.

Methods

In the present study, adult female mice of the NMRI strain (Naval Medical Research Institute, Bethesda, U.S.A.), 2–4 months of age (weight about 25 g), were used. They were fed a standard pellet diet and water *ad libitum* except when urine was collected in which case the mice were given 4 g daily of a partly synthetic diet, the composition of which was given in an earlier report (Gustafsson, Kahlson & Rosengren, 1957).

Testosterone (AB Leo, Helsingborg) was suspended in arachis oil and administered subcutaneously. Controls were injected with arachis oil only.

Identification of the yellow DNB (2,4-dinitrobenzene)-product in mouse urine

As mentioned above, urine of testosterone-treated mice contained an unidentified compound which gave a yellow product after reaction with DNFB (Henningsson & Rosengren, 1972). As a first step towards identifying the unknown substance, mouse urine was purified by ion-exchange chromatography on a Dowex 50 W-X4 column (Kahlson, Rosengren & Thunberg, 1963). A sample of the eluate was evaporated to dryness under reduced pressure and the residue was dissolved in a few drops of acetone:0·2 N HCl (1:1 by vol.) and applied to Whatman No. 1 paper. The paper was subjected to ascending chromatography for 17 h in a solvent of isopropanol:chloroform:ammonia:water (55:25:10:10; Robinson & Green, 1964). A narrow strip cut along the edge of the dried paper was sprayed with 0·2% ninhydrin in water-saturated *n*-butanol. Three distinct violet bands developed after heating the strip for a few minutes at 90° C. The bands had the following R_F-values: 0·34 (I), 0·45 (II), 0·53 (III). On spraying the strip with Pauly's diazo reagent, band II turned red.

The three areas of the main chromatogram which corresponded to the ninhydrin-positive bands were cut out and eluted with 0·2 N HCl. The eluates were evaporated to dryness and the residues were treated with DNFB. Each of the three eluates formed water soluble DNB-derivatives, which were isolated and subjected to thin-layer chromatography on silica gel plates (White, 1966). The chromatograms were developed in benzene:ethanol:ammonia (80:18:2). The R_F-values of the resulting spots were compared to those of authentic samples. It was found that the R_F-value of band I corresponded to the compound searched for, band II corresponded to histamine, and band III to 1,4-methylhistamine.

Another sample of eluate from the Dowex-50 column was chromatographed on paper as described above. The area corresponding to band I was eluted with 0·2 N HCl. The eluate was evaporated to dryness, dissolved in a few drops of acetone:0·2 N HCl (1:1) and re-chromatographed on paper for 16 h with *n*-propanol 0·2 N ammonia (3:1) as a solvent (Ames & Mitchell, 1952). Analysis of a narrow strip of the chromatogram with ninhydrin revealed only on band, R_F 0·25. Material of corresponding area of the paper was collected and re-chromatographed in a third solvent system, *n*-propanol: 1 N acetic acid (3:1). Ninhydrin treatment of a strip of the dried paper yielded one violet spot, R_F 0·15, and one brownish spot, R_F 0·26. By reaction with DNFB of eluates from appropriate areas of the main portion of the paper it was found that the band corresponding to the lower R_F-value contained the unidentified compound.

The procedure employed in detecting the unidentified compound (adsorption to Dowex-50 cation exchange resin, condensation with DNFB, positive ninhydrin reaction) suggested that it might be an amine. Incubation of mouse urine with diamine oxidase (Hog kidney, Grade II, Sigma) catabolized the compound, a finding that pointed to the unknown substance being a diamine. In order to strengthen this presumption, a large sample of mouse urine was subjected to the purification procedures to yield enough of the pertinent material to permit its identification. The isolated substance was compared to several authentic amines and was found to be putrescine since this amine could not be distinguished from the unidentified material by paper chromatography either in the systems mentioned above or in the following two systems: *n*-butanol:acetic acid:water:pyridine (15:3:12:10; Waley & Watson, 1953) and 2-methoxyethanol:propionic acid:water (14:3:3,

saturated with NaCl; Herbst, Keister & Weaver, 1958). Furthermore, the water soluble DNB-derivatives of putrescine and the unknown substance had identical R_F-values in two thin-layer chromatography systems, R_F 0·10 in benzene:ethanol:ammonia (80:18:2; White, 1966) and R_F 0·37 in *n*-butanol:acetic acid:water (60:15:25; White, 1966).

Quantitative assay of urinary putrescine

Mice were kept individually in metabolism cages that allowed urine and faeces to be collected separately. Urine was collected in 24 h samples in vessels containing 15 drops of 6 N HCl. After filtration, the 24 h samples, if kept frozen, could be stored for weeks before analysis.

On purification, after adjusting the pH to 6·5, aliquots of urine were transferred to columns of 0·3 g Dowex 50 W-X4 (100–200 mesh). The column was washed with 5 ml 0·1 M phosphate buffer, pH 6·5, and 5 ml 1 N HCl. The amines were eluted by 3 ml 8 N HCl. The eluate was evaporated to dryness under reduced pressure. The residue was dissolved in a few drops of water and treated by ascending paper chromatography for 17 h in isopropanol:chloroform:ammonia:water (55:25:10:10). The spot corresponding to putrescine was eluted in 0·2 N HCl. The eluate was evaporated to dryness. The residue was dissolved in a small volume of distilled water applied to a sheet of Whatman No. 1 chromatography paper that was subjected to electrophoresis at 7·5 V per cm for 4 h in a 0·1 M sodium citrate buffer, pH 4·3. The paper was dipped in a mixture of 1 g ninhydrin, 100 ml of acetone, 5 ml of concentrated acetic acid, and 100 mg of cadmium acetate (Russell, Medina & Snyder, 1970). The paper was dried for 90 min at 60° C and the coloured area was eluted for 30 min with 5·0 ml of water-ethanol-concentrated acetic acid (1:4:5) containing 2 mg of cadmium acetate per ml. The light absorption of the resulting solution was measured at 505 nm with a Beckman B spectrophotometer. Recovery of putrescine standards added to urine aliquots was determined and the values for putrescine are expressed in terms of μg free base/24 h and corrected for a recovery of 80%.

Determination of ornithine decarboxylase activity

Minced tissue samples were incubated in a reaction mixture containing 0·005 μCi of ^{14}C-carboxyl labelled DL-ornithine monohydrochloride (0·5 mM final concentration), 5×10^{-5}M pyridoxal-5′-phosphate, 10^{-4}M EDTA (ethylenediamine tetra-acetic acid, disodium salt), and 10^{-1}M sodium phosphate buffer, pH 7·4, and 0·2% (w/v) glucose, the total finally made up to a volume of 2·0 ml. The mixture was incubated for 3 h at 37° C in a closed vessel, the construction of which has been described earlier (Grahn & Rosengren, 1968). The reaction was stopped by tipping 1 ml of 2 M citric acid from a side arm of the incubation vessel. The expelled $^{14}CO_2$ was trapped on a 10×25 mm piece of No. 005 Munktell filter paper moistened with 100 μl of hydroxide of hyamine 10–X (1 M solution in methanol). Complete absorption of $^{14}CO_2$ was achieved by continued shaking for 45 minutes. The filter paper was then dropped into Bray (1960) scintillation mixture and its radioactivity was measured in a Nuclear Chicago spectrometer.

Determination of histidine decarboxylase activity

Histidine decarboxylase activity (histamine forming capacity, HFC), was deter-

mined by the 'pipsyl' method of Schayer as adapted for use in our laboratory (Kahlson *et al.*, 1963). The method involved the following steps: minced tissue samples were incubated for 3 h at 37° C under nitrogen in beakers containing 100 mg of tissue, 20 μg (1·25 μCi) of 2-ring-[^{14}C] L-histidine (base), 10^{-5}M pyridoxal-5′-phosphate, 10^{-4}M aminoguanidine sulphate, 10^{-1}M sodium phosphate buffer of pH 7·4 and 0·2% (w/v) glucose, the total finally made up to a volume of 1·5 ml. On completing the incubation, carrier histamine and perchloric acid were added, and the content of each beaker was filtered. Radioactive histidine was separated from radioactive histamine on an ion exchange resin (Dowex 50 W–X4, 100–200 mesh) and after converting the amine to its dipipsyl derivative the radioactivity of the histamine formed was determined at infinite thickness in a methane gas flow counter. The pipsyl samples were repeatedly recrystallized from acetone until they displayed constant radioactivity.

Results

Part of the main results and how these were obtained has, of necessity, been mentioned in the preceding **Methods** section.

Excretion of putrescine in mice during testosterone administration

The amount of putrescine excreted in the urine during testosterone administration was determined in seven mice. Before beginning the experiments, the mice were accustomed to living in metabolism cages. Urine was collected for 4 days before testosterone injections. The mice were then injected with testosterone, 0·5 mg daily for 10 days, and collection of urine continued.

Before testosterone administration, the mean putrescine excretion in 14 determinations was 23 μg/24 h; the extreme values observed were 8–65 μg/24 hours. Within 24 h after the first injection of testosterone, the putrescine excretion was found to be elevated and it gradually increased under the influence of testosterone, attaining figures about 10 times the controls. On discontinuing testosterone treat-

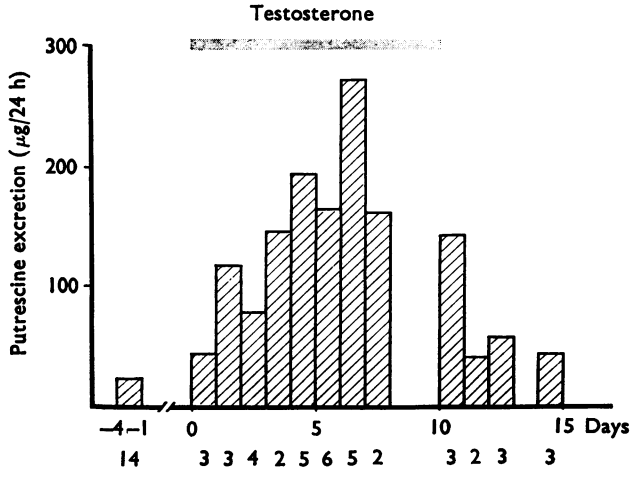

FIG. 1. Mean urinary excretion of putrescine (μg/24 h) in seven female mice before, during and after administration of testosterone, 0·5 mg daily for 10 days. The numerals in the bottom row show the number of animals examined on the day indicated, except for the four pre-treatment days when 14 determinations were done in a group of seven mice.

ment, the excretion fell steeply to the control level within 48 h or remained slightly above. These observations on putrescine excretion in urine are summarized in Figure 1.

Changes in ornithine decarboxylase and histidine decarboxylase activities in mice induced by testosterone

The kidney and the liver of mice treated with testosterone were examined for ornithine decarboxylase and histidine decarboxylase activities. Seven litters were divided into controls and treated mice, the treated ones receiving testosterone, 0·5 mg daily for 3–7 days, as stated in Table 1. The control groups were given arachis oil only.

TABLE 1. *Ornithine decarboxylase and histidine decarboxylase activities of kidney and liver in mice given 0·5 mg testosterone daily and killed on different days after the first injection, as stated*

Litter No.	Testosterone (days)	Ornithine decarboxylase (nmoles × 10) Kidney C	Kidney T	Liver C	Liver T	Histidine decarboxylase (nmoles) Kidney C	Kidney T	Liver C	Liver T
1	3	102	258	125	133	54	0·66	0·254	0·232
			100		96		0·276		0·173
2	3	75	266	13	35	15·9	0·260	0·118	0·184
			67		41		0·071		0·223
3	6	109	490	79	96	22·3	0·106	0·121	0·202
			370		74		0·084		0·130
4	6	113	430	92	72	690	165	0·167	0·223
		146	219	64	97	64	203	0·46	0·284
5	6	93	700	118	85	203	0·059	0·193	0·172
		103	1,050	83	84	53	0·078	0·150	0·192
6	7	96	63	80	75	8·5	0·243	0·117	0·144
		83	111	50	45	36	0·063	0·144	0·099
7	7	95	730	112	103	500	55	0·46	0·233
		88	—	58	104	16·8	105	0·154	0·109
		99		25		590		0·088	
	Mean	100	370	75	81	187	38	0·202	0·186
	S.D.	17·9	301	35	27·2	252·2	69·2	0·1279	0·0524
		$P<0·01$		NS		$P<0·05$		NS	

Controls received arachis oil only. The enzyme activities are expressed as nmoles of the amines formed per g wet weight of tissue in 3 hours. C=control; T=testosterone treated. The means ± S.D. given were calculated for each experimental group disregarding length of hormonal treatment.

In the liver, as seen in Table 1, the fluctuations of histidine decarboxylase activity were small among the litters and likewise between the individual controls. No detectable change in enzyme activity occurred on administering testosterone. In contrast, the kidney histidine decarboxylase showed conspicuous variations between the different litters and the individual figures within a litter. Testosterone administration produced a striking fall in the histamine formation of the kidney in each except for two experiments (litter Nos. 4 and 7). In these two experiments the control mice showed exceptionally high figures and great individual fluctuations in enzymic levels which presumably made it difficult to reveal any lowering of histidine decarboxylase activity after testosterone administration. We have no explanation of the variations of kidney histamine formation in these particular controls except that the difference between the litters may be accounted for by genetic factors. In a previous study of histamine formation in mouse kidney similar large variations were found (Rosengren, 1963).

Ornithine decarboxylase activities in the kidney and liver of the control groups showed only small fluctuations (Table 1). The capacity to form putrescine was of about the same order in the kidney and the liver. After testosterone injections no significant change of the ornithine decarboxylase activity was observed in the liver. The kidney enzyme, in contrast, was elevated, sometimes five- to ten-fold.

Discussion

The amines histamine, putrescine, spermidine and spermine are normal constitutents of most mammalian tissues. Whilst histamine has been explored since the beginning of this century, putrescine and the polyamines have only recently become the subject of concerted studies although their occurrence in human semen has been known for more than a century (see Williams-Ashman, Pegg & Lockwood, 1969; Herbst & Bachrach, 1970). Among these, putrescine appears to hold a key position in that the formation of putrescine is the first and perhaps rate limiting step in the biogenesis of polyamines in mammalian cells (Williams–Ashman *et al.*, 1969). Tissue ornithine decarboxylase activity is in part controlled by end-product repression (Tabor & Tabor, 1969); a similar situation exists in the feedback relation between histidine decarboxylase and histamine (Kahlson, Rosengren, Svahn & Thunberg, 1964).

The rat ventral prostate is a rich source of L-ornithine decarboxylase, forming putrescine and CO_2 from L-ornithine (Pegg & Williams–Ashman, 1968). In mammals synthesis of putrescine by routes other than decarboxylation of L-ornithine has not been demonstrated (see Williams–Ashman *et al.*, 1969). It has been shown in rats after orchiectomy that the levels of prostatic putrescine declined noticeably, and that these rats on treatment with testosterone displayed a significant increase in putrescine within 24 h after the injection of the hormone; the level continued to increase over the next few days after androgen treatment was commenced. A rapid rise in the activity of the prostatic ornithine decarboxylase ensued, which was doubled 6 h after giving the hormone (Williams–Ashman *et al.*, 1969). In the present study, the precise time course of elevation of ornithine decarboxylase activity in the kidney was not established.

Recently, interest has been focussed on the possibility that polyamines may play a role in the process of tissue growth and RNA metabolism. This field of study was opened by the findings that such amines in low concentrations stimulate growth of bacteria (see Tabor, Tabor & Rosenthal, 1961), and a mammalian cell line (Ham, 1964). Further work along these lines was stimulated by the pioneer work of Raina and his co-workers. As a start, it was discovered that the developing chick embryo was rich in polyamines (Raina, 1963). Next, it was found that in the regenerating rat liver synthesis of spermidine from ^{14}C-methionine *in vivo* was substantially increased within a few hours after partial hepatectomy (Raina, Jänne & Siimes, 1965, 1966; Dykstra & Herbst, 1965). Following these reports, results have shown that putrescine is formed at high rates in the regenerating rat liver, accounted for by an early increase in ornithine decarboxylase activity (Jänne & Raina, 1966, 1968; Jänne, 1967; Russell & Snyder, 1968; Fausto, 1969). In this connection it should be mentioned that in the mouse, testosterone given for two to three weeks has been reported to produce hypertrophy of various epithelial structures in the kidney cortex (Selye, 1939; Pfeiffer, Emmel & Gardner, 1940). In the present study, in the female mouse kidney following testosterone administration,

the normally high histidine decarboxylase activity fell steeply and ornithine decarboxylase activity was concomitantly elevated.

At the beginning of the experiments on polyamines as related to growth it had been demonstrated in the present authors' laboratory that a number of normal and malignant rapidly growing tissues generated high amounts of histamine (for references see Kahlson & Rosengren, 1968, 1971). Among tumour tissues, a few so far investigated have been reported to produce putrescine instead of histamine (Russell & Snyder, 1968). It would appear that in these situations putrescine substitutes for histamine in some instances of growth.

The methods for measuring putrescine employed in our previous and present studies require comment. White (1966) found that only small amounts of untreated DNFB, which is insoluble in water, occasionally appeared in the thin layer chromatograms. Consequently, the water-insoluble derivatives of DNB are likely to have in part escaped detection in the plates of our earlier study. Such derivatives are formed from several di-amino compounds, among others, putrescine (McIntire, White & Sproull, 1950). Since the unknown compound appearing as a yellow coupling product on the thin layer chromatogram has now been identified as putrescine, it would appear that the quantities previously reported to be excreted by testosterone-treated mice represent only a small fraction of the total amount of this amine actually collected. The use of an improved method for measuring putrescine in this study confirms this assumption, as is evident from Figure 1. The peak levels excreted in 24 h are roughly ten times higher than those found earlier by Henningsson & Rosengren (1972).

Organ specificity of the enzymic changes provoked by testosterone was shown by the fact that ornithine decarboxylase activity was elevated in the kidney, but not in the liver. The kidney of the mouse may serve as a useful target organ also in studying the relationship between hormones and histidine decarboxylase (Henningsson & Rosengren, 1972). The present study further suggests that this organ may provide a rewarding model in exploring the biogenesis and physiology of polyamines.

In conclusion the present experiments have revealed new actions of testosterone: the ability to alter the activity levels of two enzymes. The means by which these alterations are brought into effect are likely to remain unknown as long as the intracellular mechanism of action of gonadal steroid hormones is unknown. The action of testosterone, as here recorded, should perhaps be seen as opposed to that of oestradiol which greatly elevates the level of kidney histidine decarboxylase (Henningsson & Rosengren, 1972).

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REFERENCES

AMES, B. N. & MITCHELL, H. K. (1952). The paper chromatography of imidazoles. *J. Am. chem. Soc.*, **74**, 252–253.

BRAY, G. (1960). A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. *Anal. Biochem.*, **1**, 279–285.

DYKSTRA, W. G. & HERBST, E. J. (1965). Spermidine in regenerating liver: relation to rapid synthesis of ribonucleic acid. *Science, N.Y.*, **149**, 428–429.

FAUSTO, N. (1969). Studies on ornithine decarboxylase activity in normal and regenerating livers. *Biochim. biophys. Acta*, **190**, 193–201.

GRAHN, B. & ROSENGREN, E. (1968). Adrenaline induced acceleration of histamine formation *in vitro*, studied by two isotopic methods. *Br. J. Pharmac.*, **33**, 472–479.

GUSTAFSSON, B., KAHLSON, G. & ROSENGREN, E. (1957). Biogenesis of histamine studied by its distribution and urinary excretion in germ free reared and not germ free rats fed a histamine free diet. *Acta physiol. scand.*, **41**, 217–228.

HAM, R. G. (1964). Putrescine and related amines as growth factors for a mammalian cell line. *Biochem. biophys. Res. Commun.*, **14**, 34–38.

HENNINGSSON, S. S. G. & ROSENGREN, E. (1972). Alterations of histamine metabolism after injections of sex hormones in mice. *Br. J. Pharmac.*, **44**, 517–526.

HERBST, E. J. & BACHRACH, U. (1970). Metabolism and biological functions of polyamines. *Ann. N.Y. Acad. Sci.*, **171**, 691–1009.

HERBST, E. J., KEISTER, D. L. & WEAVER, R. H. (1958). The separation of aliphatic amines by paper chromatography or paper electrophoresis. *Archs Biochem. Biophys.*, **75**, 178–185.

JÄNNE, J. (1967). Studies on the biosynthetic pathway of polyamines in rat liver. *Acta physiol. scand.*, suppl. 300, pp. 1–71.

JÄNNE, J. & RAINA, A. (1966). Further observations on the biosynthesis of polyamines in regenerating rat liver. *Acta chem. scand.*, **20**, 1174–1176.

JÄNNE, J. & RAINA, A. (1968). Stimulation of spermidine synthesis in the regenerating rat liver: relation to increased ornithine decarboxylase activity. *Acta chem. scand.*, **22**, 1349–1351.

KAHLSON, G. & ROSENGREN, E. (1968). New approaches to the physiology of histamine. *Physiol. Rev.*, **48**, 155–196.

KAHLSON, G. & ROSENGREN, E. (1971). *Biogenesis and Physiology of Histamine*. London: Arnold.

KAHLSON, G., ROSENGREN, E., SVAHN, D. & THUNBERG, R. (1964). Mobilization and formation of histamine in the gastric mucosa as related to acid secretion. *J. Physiol., Lond.*, **174**, 400–416.

KAHLSON, G., ROSENGREN, E. & THUNBERG, R. (1963). Observations on the inhibition of histamine formation. *J. Physiol., Lond.*, **169**, 467–486.

MCINTIRE, F. C., WHITE, F. B. & SPROULL, M. (1950). The determination of histamine with 2,4-dinitrofluorobenzene. *Archs Biochem.*, **29**, 376–386.

PEGG, A. E. & WILLIAMS-ASHMAN, H. G. (1968). Biosynthesis of putrescine in the prostate gland of the rat. *Biochem. J.*, **108**, 533–539.

PFEIFFER, C. A., EMMEL, V. M. & GARDNER, W. U. (1940). Renal hypertrophy in mice receiving estrogens and androgens. *Yale J. biol. Med.*, **12**, 493–501.

RAINA, A. (1963). Studies on the determination of spermidine and spermine and their metabolism in the developing chick embryo. *Acta. physiol. scand.*, **60**, suppl. 218, pp. 1–81.

RAINA, A., JÄNNE, J. & SIIMES, M. (1965). Polyamines and nucleic acids in regenerating rat liver. Abstracts, 2nd FEBS Meeting, Vienna, A 114.

RAINA, A., JÄNNE, J. & SIIMES, M. (1966). Stimulation of polyamine synthesis in relation to nucleic acids in regenerating rat liver. *Biochim. biophys. Acta*, **123**, 197–201.

ROBINSON, J. D. & GREEN, J. P. (1964). Presence of imidazoleacetic acid riboside and ribotide in rat tissues. *Nature, Lond.*, **203**, 1178–1179.

ROSENGREN, E. (1963). Histamine metabolism in the pregnant mouse. *J. Physiol., Lond.*, **169**, 499–512.

RUSSELL, D. H., MEDINA, V. J. & SNYDER, S. H. (1970). The dynamics of synthesis and degradation of polyamines in normal and regenerating rat liver and brain. *J. biol. Chem.*, **245**, 6732–6738.

RUSSELL, D. & SNYDER, S. (1968). Amine synthesis in rapidly growing tissues: ornithine decarboxylase activity in regenerating rat liver, chick embryo, and various tumours. *Proc. natn. Acad. Sci., U.S.A.*, **60**, 1420–1427.

SELYE, H. (1939). The effect of testosterone on the kidney. *J. Urol.*, **42**, 637–641.

TABOR, H. & TABOR, C. W. (1969). Formation of 1,4-diaminobutane and of spermidine by an ornithine auxotroph of *Escherichia coli* grown on limiting ornithine or arginine. *J. biol. Chem.*, **244**, 2286–2292.

TABOR, H., TABOR, C. W. & ROSENTHAL, S. M. (1961). The biochemistry of the polyamines: spermidine and spermine. *A. Rev. Biochem.*, **30**, 579–604.

WALEY, S. G. & WATSON, J. (1953). Some peptides of lysine. *J. Chem. Soc.*, part 1, 475–479.

WHITE, T. (1966). Histamine and methylhistamine in cat brain and other tissues. *Br. J. Pharmac Chemother.*, **26**, 494–501.

WILLIAMS-ASHMAN, H. G., PEGG, A. E. & LOCKWOOD, D. H. (1969). Mechanisms and regulation of polyamine and putrescine biosynthesis in male genital glands and other tissues of mammals. *Adv. Enz. Reg.*, **7**, 291–323.

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