

essential for the communication of discrete chemical messages and the evolutionary mechanisms which are responsible for these phenomena are currently underway and will be the basis of subsequent reports.

In order to apply these results to survey and detection of the oak leaf roller infestations or to the eventual control of this pest, a mixture of several isomers might prove to be essential. In any case, it is evident that sexual communication in the oak leaf roller and perhaps other insect species is exceedingly complex and presents an interesting challenge in this area of science<sup>11</sup>.

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<sup>13</sup> Note added in proof: For a possible explanation of these results see L. B. HENDRY, J. K. WICHMANN, D. M. HINDENLANG, R. O. MUMMA and M. E. ANDERSON, *Science* 188, 59 (1975).

**Zusammenfassung.** Die früher als Bestandteile der von Abdominalextrakten des weiblichen Eichenblattrollers, *Archips semiferanus* Walker, festgestellten 21 Isomeren von Tetracetylenyl-Acetat wurden mit der Elektroantennogramm- (EAG) und der Fallenfang-Methode auf ihre Wirkung bei Männchen von *A. semiferanus* geprüft. Während alle 21 Isomere sich als EAG-aktiv erwiesen, vermochten nur 17 Männchen anzulocken. Aktivitätsvergleiche der Isomeren haben gezeigt, dass zwischen den Resultaten der beiden Test-Methoden nur eine geringe Beziehung existiert.

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## Effect of Spermine on Adenyl Cyclase Activity of Spermatozoa

The presence of large quantities of spermine (2–15 mM) in human seminal plasma has been reported<sup>1–3</sup>. However, the role of polyamine, if any, in spermatozoal motility and metabolism still remains problematical. TABOR and ROSENTHAL<sup>4</sup> have reported that addition of high concentration of spermine to spermatozoa obtained from the vas deferens of mice, rats, guinea-pigs and rabbit, produced an unusual hyperactivity characterized by rapid vibrations without forward motion. However, the specificity of the effects of spermine on sperm cell movement still remains to be clearly understood.

Recently, we have reported that maltase activity of human seminal plasma increased on addition of spermine (up to 3 mM)<sup>5</sup>. Further, it was observed that spermine decreased the utilization of fructose by spermatozoa. Studies carried out by GARBERS, FIRST and LARDY<sup>6</sup> have demonstrated that motility can be induced and prolonged in spermatozoa of several mammalian species, by cyclic nucleotides. The purpose of the present investigation was to study whether polyamines-spermine, spermidine and putrescine would affect the spermatozoa adenyl cyclase activity and consequently sperm motility.

Activation of adenylate cyclase of human spermatozoa by spermine expressed in terms of cyclic AMP produced (pmoles/mg sperm protein/10 min)

Concentration of spermine (mM)	Semen samples from fertile donors							
	A	B	C	D	E	F	G	H
—	38	165	20	13	16	49	134	75
2.9	38	185	38	48	22	78	140	<sup>a</sup>
7.6	105	195	53	57	29	71	<sup>a</sup>	222
13.4	130	215	<sup>a</sup>	127	<sup>a</sup>	88	174	258

<sup>a</sup> As the determinations were carried out in duplicate and at 3 levels of added spermine, some semen samples did not have sufficient number of spermatozoa for the test to be carried at all dose levels.

(<sup>3</sup>H) adenosine 3'-5'-cyclic monophosphate (specific activity, 20–30 Ci/mM) was purchased from Radiochemical Centre (Amersham, England). Spermine tetrahydrochloride was obtained from Sigma Chemical Co. (USA). Semen samples were obtained from 8 fertile donors (samples A to H mentioned in the Table). After liquefaction of the semen samples, the seminal plasma was separated from spermatozoa by centrifuging the semen at 800 g for 30 min. Seminal plasma was drained off and sperms were washed with 3 ml of Tris-HCl buffer, centrifuged and resuspended in the buffer. Protein content of sperm suspension was determined according to the method as described by LOWRY et al.<sup>7</sup>.

Determination of adenyl cyclase activity was carried out as follows. The incubation mixture contained sperm (adjusted to mg of sperm protein) suspended in 0.04 M Tris HCl buffer (pH 7.1), 0.033 M MgSO<sub>4</sub>, 10 mM theophylline, 1 mM adenosine triphosphate, and different concentrations of spermine as indicated in the Table. The total volume of the reaction mixture was 0.4 ml and the incubation was carried out at 37°C for 10 min. The cAMP was assayed by the competitive binding assay as described by TSANG et al.<sup>8</sup>, using bovine adrenocortical receptor protein. All the determinations were carried out in duplicates.

<sup>1</sup> A. LEEUWENHOEK (1678) as quoted by T. MANN, in *The Biochemistry of Semen and of Male Accessory Reproductive Tract* (Methuen & Co., London 1964).

<sup>2</sup> H. TABOR and C. W. TABOR, *Pharmac. Rev.* 16, 245 (1964).

<sup>3</sup> A. N. THAKUR, A. R. SHETH, SHANTA S. RAO and D. S. PARDHANAN, *Indian J. Biochem. Biophys.* 10, 134 (1973).

<sup>4</sup> C. W. TABOR and S. L. ROSENTHAL, *J. Pharmac. exp. Ther.* 116, 139 (1956).

<sup>5</sup> A. R. SHETH, G. V. SHAH and SHANTA S. RAO, *Andrologie* 6, 347 (1974).

<sup>6</sup> D. L. GARBERS, N. L. FIRST and H. A. LARDY, *J. biol. Chem.* 248, 875 (1973).

<sup>7</sup> O. H. LOWRY, N. J. ROSEBROUGH, A. FARR and R. J. RANDALL, *J. biol. Chem.* 193, 265 (1951).

<sup>8</sup> C. P. W. TSANG, D. C. LEHOTAY and B. E. P. MURPHY, *J. clin. Endocr. Metab.* 35, 809 (1972).

Results, as can be observed in the Table, indicate that basal cyclic AMP levels vary between 16 to 165 pmoles/mg sperm protein. These variations in cyclic AMP levels are comparable to those observed by GRAY, HARDMAN, HAMMER, HOOS and SUTHERLAND<sup>9</sup>. Further, our studies demonstrate that sperm cAMP levels increased several fold on addition of spermine (2.9 mM to 13.4 mM). The results presented clearly demonstrate that the physiological concentration of spermine enhances the cAMP levels considerably. GARBERS, FIRST and LARDY<sup>6</sup> and HICKS et al.<sup>10</sup> have observed that cyclic nucleotides regulate spermatozoal motility and metabolism. They have also shown that cAMP increase the oxidation of lactate, succinate and citrate by human spermatozoa. Further, the increase in oxidation metabolism resulted in increased spermatozoal motility<sup>11</sup>. TASH and MANN<sup>12</sup> have shown that concentration of cAMP in spermatozoa represents a very accurate and sensitive indicator of sperm activity. According to our findings, polyamine can activate cAMP levels of spermatozoa which provides the basis for the observation of TABOR and TABOR<sup>2</sup>, who reported activation of spermatozoal motility.

Presence of polyamines and enzymes involved in polyamine biosynthesis-ornithine decarboxylase in human cervical mucus have been detected by us (unpublished observation). RUSSELL et al.<sup>13</sup> have reported the presence of ornithine decarboxylase activity in the rat uterus. Further, these workers have shown that the enzyme level

increases on administration of estradiol. If human spermatozoa undergo capacitation, as do the spermatozoa of hamster, rabbit etc., then polyamines present in the female genital tract would have an important role to play in sperm capacitation, by increasing the spermatozoal cAMP levels. The mechanism by which polyamine activates adenyl cyclase remains to be elucidated.

**Zusammenfassung.** Zusätze physiologischer Sperminkonzentrationen (2–14 mM) zu menschlichen Spermien-suspensionen bewirken eine Steigerung der Adenylcyclase-Aktivität, wie sie durch die vermehrte Bildung von cAMP aus ATP angezeigt wird.

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<sup>9</sup> J. P. GRAY, J. G. HARDMAN, J. L. HAMMER, R. T. HOOS and E. W. SUTHERLAND, *Fedn. Proc.* 30, 1251 (1971).

<sup>10</sup> J. J. HICKS, N. PEDRON and A. ROSADO, *Fertil. Steril.* 23, 886 (1972).

<sup>11</sup> J. J. HICKS, N. PEDRON, J. MARTINEZ-MANANTOU and A. ROSADO, *Fertil. Steril.* 23, 172 (1972).

<sup>12</sup> J. TASH and T. MANN, *J. Reprod. Fert.* 35, 591 (1973).

<sup>13</sup> D. H. RUSSELL and R. L. TAYLOR, *Endocrinology* 88, 1397 (1971).

## Cerebral Uptake of Noradrenaline in vitro; Occurrence of Different Uptake Systems and Effect of Partial External Sodium Substitution

Exogenous noradrenaline (NA) is actively accumulated into cerebral cortex slices against a concentration gradient<sup>1,2</sup>. NA is inactivated by re-uptake into the pre-synaptic endings after its release from synapses. SNYDER et al.<sup>3</sup> have studied NA accumulation by different brain structures incubated in oxygenated physiological media. They found that there was only a single NA uptake system with different kinetic parameters for different structures, except in the case of the cerebellum which did not show saturable accumulation<sup>3</sup>.

Cerebral tissue have different affinities for the uptake of dopamine<sup>4</sup>, serotonin<sup>5</sup>,  $\gamma$ -aminobutyric acid<sup>6</sup> and choline<sup>7</sup>. We have studied NA transport in cerebral cortex slices with a larger range of concentrations than those used by SNYDER et al.<sup>3</sup>.

We also studied the effects of Na<sup>+</sup> deficient medium on NA cerebral transport as the uptake of these other compounds is dependent upon the concentration of ions in the medium<sup>8</sup>.

**Experimental procedure.** Male Wistar rats, weighing 200–250 g were used. The preparation and incubation of the cerebral slices were carried out as described previously<sup>9,10</sup>. Slices (60–70 mg) were pre-incubated for 30 min in Krebs-Ringer bicarbonate saline which was also used for incubation; it had the following composition, (mM), NaCl, 124; KCl, 5; KH<sub>2</sub>PO<sub>4</sub>, 1.24; MgSO<sub>4</sub>, 1.3; CaCl<sub>2</sub>, 2.8; NaHCO<sub>3</sub>, 26; glucose, 10; the pH was kept at 7.4 by continuous saturation with 95% O<sub>2</sub>: 5% CO<sub>2</sub> mixture. When Na<sup>+</sup> deficient solutions were used, the NaCl and NaHCO<sub>3</sub> were replaced by choline chlorydrate and choline bicarbonate, respectively (Sigma Co., St. Louis, USA).

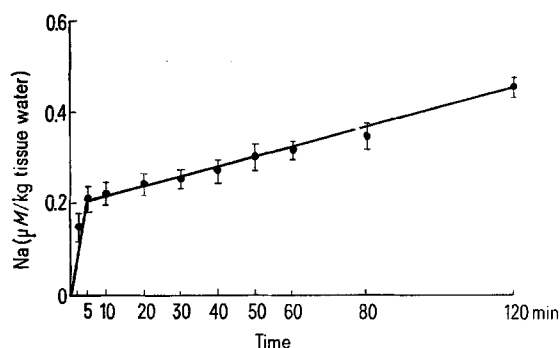


Fig. 1. Kinetics of the accumulation of DL-<sup>3</sup>H-NA at a concentration of 0.2 μM/l at 37°C in a normal Krebs Ringer medium. Each point is the mean of 10 slices, with the vertical bars representing one standard deviation.

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<sup>2</sup> B. HAMBERGER and D. MASUOKA, *Acta Pharmac. Toxic.* 22, 363 (1965).

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