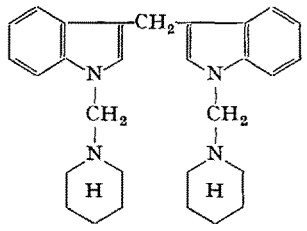
changes in the indole structure, effective central depressants might be prepared. In the following paper, we give an account of our work in this direction, begun in 1961, in which we found N,N'-di(piperidino-methyl)-3,3'-diindolylmethane (DIM)[9] to be one of the most potent among the several compounds synthesized. Compounds of similar structure have already been dealt with, but no pharmacological results have yet been published[10]. Our detailed chemical and pharmacological results will be reported on at a later date[11].



base m.p. 132°C

The compound is a white, crystalline substance. It is not soluble in water, but absolutely pure 1–2% aqueous solution can be prepared by the use of Tween 80.

Toxicity in mice is (reading the results at 48 h in an environment of 25°C) LD_{50} = 400 mg/kg s.c., or LD^{100} = 600 mg/kg s.c.; in cats (72-h result) it is LD_{100} = 25–30 mg/kg i.p.

The most remarkable property of DIM has been found to be that even when administered in low doses (3–5 mg/kg i.p.) it causes catalepsy, hypothermia and a reduction of metabolic rate in rats, mice, rabbits, cats and dogs. The effect develops slowly and lasts 24–36 h. The cause of the lowering of the body temperature is a paralysis of the thermoregulatory mechanism; namely on exposure to cold, the metabolic rate of the DIM-treated rats does not increase; on the contrary, it decreases. However, the hypothalamus remains directly stimulable chemically with Amphetamine.

As injected intravenously in a dose of 0.3 mg/kg into cats anaesthetized with chloralose-urethane, DIM reduces respiratory rate and volume and causes a lasting vasodepression. It inhibits the carotid pressor reflex, but the vasomotor centre remains responsive to CO_2. DIM possesses no atropine, antihistaminic or anti-adrenaline effects. It does not paralyse the autonomic ganglia. It blocks the polysynaptic spinal crossed extensor reflex, but has no influence on the homolateral flexor and patella reflexes.

DIM is a potent analgesic agent. Its effect is 35% stronger and more durable than that of morphine. It potentiates ether and Evipan anaesthesia. There is a very marked synergism of potentiating nature between Chlorpromazine and DIM. When administered together, a full effect is produced by 15% of the original effective doses of the two compounds. Animals treated with DIM + Chlorpromazine alone can be subjected to painful operations without any other drug treatment.

As determined in waking cats and rabbits, DIM synchronizes cerebral electrical activity and inhibits the cortical and subcortical desynchronization in response to painful stimulation[12].

Zusammenfassung. Ein neues Diindolyl-methan-derivat, das N, N'-di(piperidino-methyl)-3, 3'-diindolyl-methan, wurde synthetisiert; es verursacht im Tierversuch schon in Dosen von 3–5 mg/kg einen langanhaltenden zentralen Depressoreffekt.

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[9] S. FÖLDEÁK, B. MATKOVICS, and J. PÓRSZÁSZ, Hung. Pat. 150,911 (1963).
[10] J. THESING and P. BINGER, Chem. Ber. *90*, 1419 (1957).
[11] J. PÓRSZÁSZ, S. FÖLDEÁK, B. MATKOVICS, and K. PÓRSZÁSZ-GIBISZER, Acta physiol. hung., to be published.
[12] This work has been supported by grants from the Chemical Works of Gedeon Richter Ltd., Budapest.

Specific Gravity of Epididymal and Ejaculated Bull Spermatozoa and of their Parts

Specific gravity in ejaculated bull spermatozoa was studied by LINDAHL and KIHLSTRÖM[1] by centrifugation in umbradil-methylglucamine-salt solutions of different specific gravity. They arrived at the general conclusion that the process of physiologic ripening of the sperms implies a rise in gravity. Later on KIHLSTRÖM[2], using the same method, demonstrated that free heads of ejaculated spermatozoa obtained by shaking sperms with glass beads show a much higher specific gravity than the corresponding fragments consisting of mid-pieces with tails and protoplasmic drops.

In the course of a chemical study on bull spermatozoa it appeared desirable to obtain isolated heads and midpieces with tails in fairly pure suspensions and in sufficient amounts. Sonic treatment followed by centrifugation in specific weight gradients gave fairly good results. Preliminary experiments aiming at the exploration of suitable conditions for this procedure produced some data of general interest on the specific gravity of bull sperms. These will be described in the present communication.

Material and methods. Epididymal and ejaculated spermatozoa were obtained from bulls of Swedish Red and White cattle. The former were prepared according to HENLE[3] from the testes of slaughtered bulls within 1 h after death. The suspension was stored at room temperature. Ejaculated spermatozoa were obtained from semen collected at the Artificial Insemination Centre at Enköping by means of an artificial vagina, cooled immediately in the general way to 5°C and transported undiluted at this temperature to this laboratory. The mate-

[1] P. E. LINDAHL and J. E. KIHLSTRÖM, J. Dairy Sci. *35*, 393 (1952).
[2] J. E. KIHLSTRÖM, Arkiv Zool. *11*, 569 (1958).
[3] W. J. HENLE, Immunol. *34*, 325 (1938).

Specific gravity of entire epididymal and ejaculated spermatozoa and of heads and mid-pieces with tails

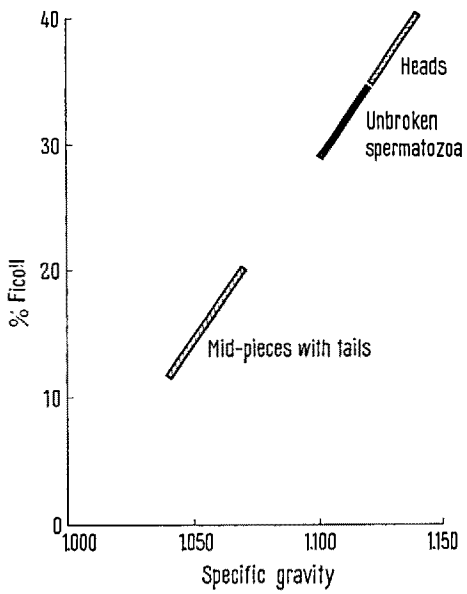
	Not treated with ultrasonics		Treated with ultrasonics	
Epididymal spermatozoa	Unfragmented	1.100–1.125	Unfragmented	1.100–1.120
			Mid-pieces with tails	1.040–1.070
			Heads	1.120–1.140
Ejaculated spermatozoa	Unfragmented	1.240–1.334[3]	Unfragmented	1.210–1.330
	Mid-pieces with tails	1.045[2]	Mid-pieces with tails	1.040–1.070
	Heads	1.276[2]	Heads	1.250–1.350

rial was used within 24 h after collection. The Ringer-phosphate of MANN[4] was used as medium for both kinds of cells.

The fragmentation of spermatozoa was performed with the aid of an ultrasonic disintegrator (MSE, Mullard, type 7680/3), the cells being subjected to 20 kc/s for 10 sec. In this way about half of the cells were broken between the head and the mid-piece, but the frequency of mid-pieces wirh tails was always less than that of isolated heads in the treated suspensions[1].

Specific weight gradients of the synthetic polysaccharide Ficoll (Pharmacia, Uppsala, Sweden), with an average molecular weight of about 400.000, were obtained by layering solutions in the above-mentioned Ringer solution. The following percentages of Ficoll were used: 5, 10, 12, 14, 16, 17, 18...50%, the interval between 18 and 50% containing all whole numbers. The dimensions of the centrifuge tubes containing the Ficoll gradient were 105 × 11 m.m. The heights of the layers of the different Ficoll solutions were 3.0–3.4 mm.

Centrifugation at 1000 *g* for 12–15 min brought the cells and the cell fragments into equilibrium with the gradient and gave reproducible results. The distribution of cells and cell fragments along the gradient was established by cautious withdrawal of the layers of different concentrations of Ficoll with a fine pipette und examination of several samples from each layer under the microscope. This was facilitated by marking the original limits between the layers with fine lines upon the outside of the tube.



Distribution of unbroken spermatozoa, heads, and mid-pieces with tails along the Ficoll gradient. Fragmentation with ultrasonics.

Results and discussion. The Figure gives the results of a typical experiment with ejaculated spermatozoa after treatment with ultrasonics. The mid-pieces with their tails have a specific density which very markedly falls below those of the entire sperms and of the isolated heads. These two fractions are also well separated in the gradient although the difference between their specific densities is small. The purity of the three samples was estimated from counts in a Bürker chamber (about 500 cells or fragments counted) to 90% for the sample containing the entire spermatozoa and 98% for the two other ones. As seen from the Table, unfragmented *epididymal* sperms show just the same relationship to the gradient and thus the same specific gravity whether treated with ultrasonics or not. It might therefore be concluded that the ultrasonic treatment used has no influence upon the specific gravity itself, nor that it has changed the permeability of the cells in such a way as to permit the polymer forming the gradient to penetrate into the interior of the cells. The present values for unfragmented *ejaculated* sperms agree well with those presented by LINDAHL and KIHLSTRÖM[1], and our values for heads and mid-pieces with tails from *ejaculated* spermatozoa are well in keeping with those published by KIHLSTRÖM[2]. This is of importance as the earlier determinations were performed with a substance of much lower molecular weight, thereby producing much higher osmotic pressures and risks for penetration into the cells.

In conclusion, the data in the Table may be summed up in the following way. There is a marked difference in specific gravity between spermatozoa taken from the epididymidis and ejaculated spermatozoa. This increase is localized to the head, whereas no change could be observed in the density of the mid-pieces with tails, the specific gravity of which is much lower than that of heads already in the epididymal spermatozoa.

Zusammenfassung. Es wird ein auffallender Unterschied im spezifischen Gewicht von epididymalen und ejakulierten Bullenspermatozoen durch Gleichgewichtszentrifugierung in Ficollgradienten festgestellt. Ähnliche Versuche mit Kopf- bzw. Mittelstück-Schwanz-Fragmenten, aus ejakulierten Spermien mittelst Ultraschallbehandlung erhalten, zeigen bei Vergleich mit früheren Angaben, dass diese Veränderung nur den Kopf betrifft.

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Institute of Zoophysiology, Uppsala (Sweden), October 9, 1964.

[4] T. MANN, *The Biochemistry of Semen* (London 1954).