animals still on the 2-hour schedule but without injections) that the drug groups were eating as much as the controls. Whether this phenomenon was a direct, prolonged, drug effect, or the result of conditioned suppression of food intake, is not known. Conditioning could have occurred through pairing of the injection with presentation of food. This is an interesting hypothesis and one worth further investigation.

In previous work(1) investigating similarities between LSD-25 and amphetamine, rats injected with these substances showed increased speed in running to escape shock and increased incidence of avoidance of shock. The present work demonstrates still another similarity between the 2 drugs, although amphetamine was not investigated here since its anorexic effects are well known.

These present studies indicate that for assay of LSD-25, food intake is a reliable and sufficient dependent variable. They also serve as a warning on the use of food reinforcement in psychological testing of drug effects.

Summary. Suppression of food intake in the rat was shown to follow injection of LSD-25 and this effect was determined to be dose dependent. When the effects of 10 daily injections of the drug were investigated, a suppression of food intake on each day indicated a lack of development of complete tolerance. These data show that caution is needed in interpretation of the behavioral effects of LSD-25 where such behavior is dependent upon food reinforcement.

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The Zinc Content of Rat Sperm Cells from Ejaculate, Vas, Epididymis and Testis.* (26926)

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No biological role is known for the high concentrations of zinc in many tissues of the male mammalian reproductive tract, including human prostate(1), ejaculate(2) and sperm cells(3), canine prostate(4) and prostatic fluid(5), rat dorsolateral prostate and rabbit prostate(6). By mating male rats after ligation of the dorsolateral prostate, Gunn and Gould(7) showed that the zinc normally released by this gland is not needed for rat fertility or fecundity. If prostatic fluid were the sole source of zinc in the rat ejaculate, their data would virtually rule out a role for (male) zinc in rat fertilization. However, Wetterdal's data(8) suggest that there is another source of zinc. After injecting zinc-65 into rats he found high activity in the testes and an activity peak which traveled through the sperm transport system along with the spermatozoa that had been maturing in the testes at time of injection. A high concentration of zinc in rat sperm cells, independent of the prostatic fluid, is confirmed in the present paper by direct measurements on spermatozoa taken from ejaculates, vas, caudal epididymis and testis.

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Methods. Ejaculated sperm cells were obtained from Wistar albino rats with electrical stimuli of 0-2 volts at an alternating current of 30 cycles per second, applied through a rectal probe(9). After ejaculation each rat was sacrificed and sperm cells were collected from the vas, caudal epididymis and testis of one side of the animal. Ejaculated copulatory plugs were obtained with peak voltages exceeding 2 volts; at lower peak voltages plugs do not form. The other tissues mentioned below were removed after sacrifice without prior ejaculation.

Sperm cells collected into Ringer's solution from ejaculates, vas and epididymis were washed in Ringer's and in water and then centrifuged at 2200 rpm (1000 g) for one hour. The sediments were placed on thin nylon films and vacuum-dried prior to zinc assay. Microscopic observations did not reveal any damage to the sperm cells (other than loss of motility) at any stage of the procedure preceding desiccation.

To obtain testicular sperm cells the testicular contents were first squeezed into Ringer's solution. After one hour at room temperature, most of the unwanted cells including immature sperm cells were settled at the bottom of the solution while the mature sperm cells, having become motile, were distributed throughout. The decanted solution was then centrifuged, yielding a residue which was predominantly sperm cells with some cellular contamination. This residue was washed in water, centrifuged again and prepared for zinc assay as described above.

Vas, dorsolateral and ventral prostates and other soft tissues were homogenized after excision in glass tube-and-pestle type tissue grinders. Homogenate samples were then mounted and dried as above.

As recommended by Sandell(10), water and Ringer's solutions were prewashed in a dilute solution of diphenylthiocarbazone ("dithizone") in carbon tetrachloride and rinsed in carbon tetrachloride to remove zinc contamination. Glassware was freed of zinc contamination by the same procedure, with an additional rinse in zinc-free water.

Zinc concentrations in the dried specimens were measured by a non-dispersive X-ray fluorescence instrument developed in this laboratory(11). Nearly monochromatic X-rays impinge on each specimen. A detector records simultaneously the scattered X-rays as an index of mass and the zinc X-rays as an index of zinc content. Zinc concentration (expressed in this paper as milligrams of zinc per gram of dried specimen) is obtained directly from these and calibration data. Sensitivity is high enough for convenient assays of sub-milligram specimens containing as little as 10^{-9} grams of zinc. Assays were generally run long enough to leave a probable statistical error of about 5%.

The adequacy of the zinc assay technic is shown in Table I, which summarizes a recovery experiment in which aliquots of a rat liver homogenate (7.5 g of rat liver in 37.5 cc of water) were supplemented with known amounts of zinc and copper sulfate dissolved in water. Copper was added because it is the one element which might be expected to interfere; a correction for this element, when necessary, is based on data obtained simultaneously from a second, suitably filtered detector. It is apparent that the method is accurate throughout the concentration range

TABLE I. Recovery Experiment.

Tube #	Added, cc	Added, dry mg	Added Zn, μg	Added Cu, μg	Nominal,* mg Cu/dry g	Nominal,* mg Zn/dry g	Measured, mg Zn/dry g
1	.99	.00	.0	.0			.097
2	.99	.06	25.0	.0	.038	.330	.328
3	.99	.25	100.	.0	.038	1.030	1.035
4	.99	.49	199.	.0	.038	1.950	1.980
5	1.98	.12	25.0	25.0	.280	.330	.334
6	1.98	.31	25.0	100.	.980	.330	.316

* Calculation of "nominal" concentrations is based on the data in the table and on measurements indicating that each aliquot, before supplementing, contained 107 dry mg, 10.4 μg of Zn and 4.1 μg of Cu in a volume of 2.50 cc.

TABLE II. Milligrams of Zinc per g of Dried Sperm Cells from Various Rat Tissues.

	Source of sperm cells			
Rat #	Testis	Caudal epididymis	Vas	Ejaculate
1	.33	.99	1.22	.79
2	.46	1.36	1.19	.96
3	.59	1.29	1.43	(lost)
4	.25	1.23	1.13	.38
5	.22	1.11	1.48	.85
6	.46	1.11	3.32	1.48
Avg	.39	1.18	1.29*	.89

* Rat #6 is omitted from the vas avg.

encountered in this report, and that the method of correction for copper is successful.

Results. Tables II and III list zinc concentrations in rat sperm cells taken from various sites, and in a variety of rat tissues.

Comparison of Tables II and III shows that an extraordinary concentration of zinc occurs in rat sperm cells independently of any contribution from prostatic or other fluid in the ejaculate.

Concerning Table II certain technical points should be noted. The most reliable values are for the vas and epididymal sperm cells. Microscopic inspection of the centrifuged material from these sources showed virtually no contamination. On the other hand, as might be expected, debris accompanied the centrifuged sperm cells from ejaculates and testes. This contamination may explain fully the lower average zinc level and higher variation in the ejaculate specimens. The contamination of the testicular material is at least as great, but there is also the possibility that the testicular sperm cells have not yet reached their ultimate zinc levels.

We do not know the reason for the odd high value for the sperm cells from the vas of animal #6; presumably the sample was inadvertently contaminated.

In order to see if the zinc levels remained unchanged through the periods of sedimentation and washing by centrifugation, spermatozoa were left at room temperature in water for extra periods up to one hour and in Ringer's solution for extra periods up to 3 hours before processing. Aliquots taken at different times during these periods showed no change in zinc levels. Repeated washings in water also had no effect.

In another "control" test to ascertain zinc retention during processing we compared the zinc level of the entire material squeezed from the vas with the level of centrifuged and washed sperm from the vas. The zinc level of the total material, comprising mainly sperm cells and vas fluid, was about 60% of the level of the processed sperm cells. The difference is due presumably to non-sperm material in the "total" specimen. The result certainly does not suggest any loss of zinc in processing of the spermatozoa.

Zinc was assayed also in specimens produced under 2 sets of electroejaculation conditions. In the copulatory plug produced with a peak stimulus above 2 volts concentration averaged 0.02 mg zinc/dried g. In the much smaller fluid specimen produced at lower voltage, after centrifugal removal of the sperm cells, concentration was 0.5 mg zinc/dried g.

For comparison, assays were run also on one canine electroejaculate. An aliquot of the whole ejaculate had 1.6 mg zinc/dried g and an aliquot after sperm cell removal (leaving only prostatic fluid) had 1.7 mg zinc/dried g. These 2 values are not significantly different. The average value of canine prostatic fluids is slightly less than 1.7 for a series run in our laboratory and to be published elsewhere, but the numbers 1.6 and 1.7 are listed in Table IV where the significance lies in their close similarity.

Discussion. Zinc is essential to many metal-

TABLE III. Milligrams of Zinc per g of Dried Rat Tissue.

Tissue	Present work*	Reference 3
Brain		.07
Epididymis	.22	.22
Heart	.10	.08
Kidney	.13	.11
Liver	.12	.11
Lung		.09
Muscle		.05
Pancreas	.07	.10
Dorsolateral prostate	1.00	.89
Ventral "	.04	
Seminal vesicle	.09	
Spleen	.18	.09
Testis	.22	.21
Vas	.16	

* Prostate and vas: Avg of 6 animals. Other tissues: Only one animal.

TABLE IV. Zinc Concentrations (mg/Dried g) in Rat, Canine and Human Prostatic Tissues, Prostatic Fluids and Ejaculates.

Species	Material: Prostatic tissue	Prostatic fluid	Ejaculate
Rat	.60*[6]	.5? †	.02‡
Dog	1.2 [4]	1.6	1.7
Man	.9 [1]	7.2[5]	3.0[2]

* Dorsolateral prostate; the value for ventral is much less.

† Electroejaculate produced at low voltage, after sperm cell removal. Volume and zinc level suggest that this material is prostatic fluid.

‡ Electroejaculate produced with a peak stimulus above 2 volts.

loenzymes(12). Its concentrations in most soft tissues are in the neighborhood of 0.1 mg/dried g(13). The much higher zinc levels in certain parts of the mammalian reproductive tract suggest a special role for the element in fertilization.

As evidence against such a role Gunn and Gould(7) recently proved, by ligation of the dorsolateral prostate, that the presumably zinc-rich prostatic fluid is superfluous for rat fertility. As they noted however, any special need for zinc in the ejaculate may be met from a different source in the rat. Since an independent source of concentrated zinc is now established by our observations (Table II) on rat testicular, vas and epididymal sperm, the possibility of a role of high zinc concentrations in fertilization is still open.

If zinc is important in ejaculates (perhaps in sperm cells), the prostatic zinc contribution may be more important in dogs and in human beings than it is in rats. Table IV shows that canine and human prostatic fluids are more effective than rat prostatic fluid in raising the zinc level of the whole ejaculate. Canine prostatic fluid is not diluted in the ejaculate by contributions from other accessory sex glands, and human prostatic fluid supplies a truly extraordinary zinc concentration; on the other hand rat prostatic fluid is such a small fraction of the total rat ejaculate (14) that the zinc level in the latter is quite low. With respect to zinc metabolism, Table IV supports Gunn and Gould's view that the rat dorsolateral prostate is archaic and indicates that the prostate gland may be more important in dogs and human beings.

Summary. Zinc concentrations are unusually high in rat spermatozoa taken from testis, caudal epididymis and vas, as well as from the ejaculate. This finding supports the view that zinc may play a role in rat fertilization, despite the fact reported by others that the zinc-rich prostatic fluid is not necessary for fertility in that species.

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