and least slope reasonably possible be drawn that "b" will have a maximum possible value of approx. 48%, and a minimum value of approx. 18%.

Discussion. Since the chromosomes appear to constitute the bulk of the content of the spermatozoon, it appears from this result that they must partake in the osmotic swelling of the cell. If the chromosomes did not swell, the value obtained for "b" would be very high, of the order of 90% or more, judging from the standard cytological pictures of sperm heads. In so reacting to hypotonic solutions, the chromosomes display an osmotic pattern shared by other formed elements within the nucleus and the cytoplasm, viz., the nucleolus, and the diverse types of granules which undergo osmotic volume changes. The value of 72% obtained by Hamburger for frog sperm may be due to a species difference or to the differing circumstances, alluded to in the introduction, in which the experimental determinations were carried out.

Churney[9] studied the swelling of the germinal vesicle in immature eggs of *Arbacia punctulata* and *Nereis limbata*. Unfortunately, no calculations of osmotically inactive fraction were given. He concluded that "the experiments failed to reveal any appreciable amount of osmotically inactive material in either of the nuclei." An interesting observation was also briefly reported in a footnote to the effect that "the giant chromosomes of the salivary gland cells of *Sciara coprophila* and of *Chironomus sp.* swell and shrink reversibly in anisotonic solutions." Quantitative data were not furnished.

Summary. A. The applicability of the Boyle-van't Hoff law to the swelling of spermatozoa of the sea urchin, *Arbacia punctulata,* in hypotonic solutions of effective concentrations varying from 42.4 to 88.5% sea water was investigated. Cell volumes were determined by centrifuging in small capillaries in an air turbine at 20,000 × gravity.

B. In the range of dilutions employed, the Boyle-van't Hoff law appeared to be followed. The "osmotically inactive fraction" ("b") averaged 33%. Thus the sperm head, which is chiefly chromosomal material, has a non-swellable volume which does not differ greatly from that of certain other cells. The results indicate that the chromosomes take part in the osmotic swelling of the cell.

9 Churney, L., *Biol. Bull.*, 1942, **82**, 52.

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An Investigation of Transmethylation from N[1]-Methylnicotinamide

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With the discovery that nicotinamide is methylated in the animal body to give N[1]-methylnicotinamide[1] it became of interest to know whether this product is a methyl donor in the biological synthesis of N- and S-methyl compounds. One approach to this problem has been the study of the lipotropic activity of N[1]-methylnicotinamide when added to a methyl-deficient diet. Najjar and coworkers[2,3] have presented data to show that N[1]-methylnicotinamide has some lipotropic action in the rat, whereas Handler and Dubin[4] found in a similar experiment that it had no activity in preventing the development of fatty livers.

We wish to report here an experiment of

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1 Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1943, **150**, 395.

2 Najjar, V. A., and Deal, C. C., *J. Biol. Chem.*, 1946, **162**, 741.

3 Najjar, V. A., and Ratcliffe, I. M., *Bull. Johns Hopkins Hosp.*, 1947, **80**, 142.

4 Handler, P., and Dubin, I. N., *J. Nutrition*, 1946, **31**, 141.

TABLE I.
Feeding Experiment with N1-deuteriomethylnicotinamide (86.0 Atom % D in the Methyl Group).

Rat No., Sex	Change in body wt g	Total amt of test compound ingested mg	Deuterium content of isolated compounds: Choline chloroplatinate atom %	Deuterium content of isolated compounds: Creatinine potassium picrate atom %
13 ♂	76-86	720	0.00 ± .09	0.00 ± .07
14 ♂	79-94	840	0.06 ± .13	0.00 ± .06

direct nature testing whether N[1]-methylnicotinamide can function as a methyl donor in the body by labeling the methyl group of this compound with deuterium. N[1]-deuteriomethylnicotinamide chloride was synthesized and fed to 2 growing rats for 6 days. At the end of this time the rats were killed and the body choline and creatine isolated. Analysis of the isolated compounds showed that no deuterium was present indicating that no transmethylation from N[1]-methylnicotinamide to choline or creatine had occurred.

Experimental. Synthesis of N[1]-deuteriomethylnicotinamide chloride ($CD_3N(Cl)$-$C_5H_4CONH_2$). This compound was prepared from deuteriomethyl iodide[5] and nicotinamide by the method of Karrer *et al.*[6] Two preparations were made, and weighed amounts of the two were mixed for the feeding experiment. For the determination of the deuterium content, the compound was burned, the water collected, purified, and the D_2O:H_2O ratio therein was measured by the falling drop method.[7] From the results obtained it was calculated that the compound contained 86.0 atom % deuterium in the methyl group.

Analysis. N[1]-deuteriomethylnicotinamide chloride.

Preparation 1. Calculated,† C 48.0; found, C. 47.9.
Preparation 2. Calculated, C 48.0; found, C 47.7.

Feeding Experiment. Two young littermate male rats were used. During a control period of 3 days they were fed *ad libitum* a diet of the following percentage composition: casein 18, sucrose 58, hydrogenated vegetable oil 19, corn oil 1, Osborne and Mendel salt mixture 4. In addition 100 g of the diet contained 20 mg choline chloride, 2 mg each of thiamine hydrochloride, riboflavin, pyridoxine hydrochloride, and nicotinic acid, 4 mg calcium-*d*-pantothenate, 4000 U.S.P. units vitamin A, 400 U.S.P. units vitamin D, and 1 mg α-tocopherol acetate.

After the 3-day control period, 2% N[1]-deuteriomethylnicotinamide chloride was put in the diet replacing an equivalent amount of sucrose. The 2 rats were pair fed. On the 6th day of feeding the test compound, rat 13 failed to eat. It was therefore killed, and autopsy showed that the kidneys were hemorrhagic. The kidneys of rat 14, which was killed at the same time, appeared to be normal. The changes in body weight and the amounts of N[1]-deuteriomethylnicotinamide chloride ingested are given in Table I.

Creatine was isolated from the carcass of each rat as creatinine potassium picrate,[5] the purity of which was determined colorimetrically by the Jaffé reaction. Choline was isolated as choline chloroplatinate.[5]

Analysis. Choline chloroplatinate.
Rat 13—Calculated, Pt. 31.7; found, Pt. 31.7.
Rat 14—Calculated, Pt. 31.7; found, Pt. 31.5.

The isolated compounds were analyzed for deuterium by the method mentioned above, and the values obtained are given in Table I.

Discussion. The isolated choline and creatine contained no deuterium. Thus, under the conditions of this experiment, there was no detectable transmethylation from N[1]-methylnicotinamide to choline or creatine.

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[6] Karrer, P., Schwarzenbach, G., Benz, F., and Solmssen, U., *Helv. Chim. Acta*, 1936, **19**, 811.

[7] Cohn, M., in Wilson, D. W., *Preparation and Measurement of Isotopic Tracers*, J. W. Edwards, Ann Arbor, 1946, p. 51.

† Calculated values are based on an increased molecular weight due to the deuterium in the molecule.

From the work of Handler and Dann,[8] and Perlzweig, Bernheim, and Bernheim,[9] as well as several more recent studies, evidence has accumulated to indicate that nicotinamide is methylated in the body by a transfer of methyl groups from a methyl donor. It would appear from the results of the present experiment as well as the experiment of Handler and Dubin[4] that this transfer must be an irreversible process. In this respect N^1-methylnicotinamide would be similar to creatine.[10]

It has been demonstrated in several instances that a compound may be inactive as a methyl donor and yet active as a lipotropic agent or in the prevention of hemorrhagic kidneys.[11] With regard to the latter, we found N^1-methylnicotinamide to be inactive. In preliminary experiments which we had carried out, we had put some young rats on a purified diet containing 2% N^1-methylnicotinamide with no added choline, and these rats died in about a week from hemorrhagic kidneys. With regard to lipotropic action, Handler and Dubin[4] found that N^1-methylnicotinamide did not prevent the development of fatty livers. On the other hand, Najjar and coworkers[2,3] observed some lipotropic action from the compound. They pointed out[3] that the slight lipotropic action which they observed might be ascribed to one of a number of factors, for example, a sparing action of the N^1-methylnicotinamide on the methionine and choline in the diet which they used.

It will be noted that a small amount of choline was present in the diet during the period of feeding N^1-deuteriomethylnicotinamide in the present experiment. The choline was included in an attempt to forestall termination of the experiment by loss of the animals from severe hemorrhagic kidney involvement. If transmethylation from N^1-methylnicotinamide to choline could take place one would expect the deuteriomethyl group to appear in the isolated choline even in the presence of some choline in the diet. This assumption is based on the fact that in several experiments carried out in this laboratory, transmethylation from deuteriomethionine to choline has been found to occur even when choline was present in the diet.[12]

It is of interest that trigonelline, N-methylnicotinic acid betaine, has likewise been shown to be inactive as a methyl donor.[8,11]

Summary. N^1-methylnicotinamide labeled in the methyl group with deuterium was fed to two rats for 6 days. Analysis of the body choline and creatine of these rats for deuterium showed that there was no detectable transmethylation from N^1-methylnicotinamide to choline or creatine during this period.

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