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# A Study of Taste and Smell of Heavy Water (99.8%) in Rats[1] (39466)

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This remarkable hydrogen isotope, deuterium oxide, constitutes one part in 5000 of all ordinary water no matter where found in the world. How and when it ever became incorporated in ordinary water is not definitely known. But it has been assumed that this water first appeared at the time of the great "Bang" of the universe (1).

Chemically, deuterium differs from hydrogen in that it has a mass of two. Physically, heavy water differs from ordinary water in a number of properties; for instance, according to Thompson (2) it is denser; it freezes, melts, and boils at higher temperatures; it is more viscous; it is a poorer solvent. It looks, however, exactly like ordinary water.

In the present experiments rats were given a choice of distilled water and heavy water (99.8%) to determine whether they recognize a difference between them, and if so, on what basis—taste, smell, or other characteristics?

*Method.* Cages used for these experiments were made of 1-cm wire mesh 19 × 32 × 25 cm (3). Graduated inverted 100-ml bottles fitted into holes placed at symmetrical positions at either side on the front of the cage. Food was available *ad lib.*[2]

To eliminate differences in temperature, supplies of distilled water and heavy water were kept at room temperature at all times.

*Do rats drink heavy water (99.8%) as freely as distilled water?* For this control experiment only one bottle was used. For 15–20 days this bottle was filled with distilled water. Records were made daily of the intake. When the intake had reached a fairly constant level, heavy water (99.8%) replaced distilled water and daily records were made as before.

Eighteen rats were used.

*Results.* In Fig. 1A for a rat, ordinates show fluid intake in milliliters; abscissas show time in days. Intake of distilled water was measured for 22 days. During the last 10 days the intake averaged 27 ml. Then heavy water replaced distilled water. On the first day the rat drank the heavy water just as freely as it had the distilled water. From then on it drank progressively less each day until its death on the twelfth day. Figure 1B shows the record of a rat that on the first day drank more heavy water than it had previously been drinking distilled water. It drank progressively less each day and died on the seventh day. The 16 other rats gave similar records. The 18 rats lived an average of 14.0 days on heavy water. On first exposure the rats certainly did not show any aversion to heavy water.

*To determine whether rats can tell the difference between heavy water and distilled water and at what concentration.* For this experiment I used the "preference threshold" technique which had been widely used in the study of reactions of rats to various nutritive substances (sodium chloride (3), five common sugars (4), alcohol (5)) and toxic substances (arsenic trioxide (6), phenylthiocarbamide (7), thallium sulfate (6)). In all instances, this technique gave sharply defined recognition thresholds.

For this technique the afore mentioned rectangular cage held two graduated inverted 100-ml bottles (made as nearly identical as possible), one at each side in symmetrical positions. These two bottles were filled with distilled water each day until the intake from each one had reached a fairly constant level. In most instances a rat consistently drinks more from one bottle than

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[2] Graham flour, 72.5%; skimmed milk powder, 10.0%; casein, 10.0%; butter, 5.0%; calcium carbonate, 1.5%; sodium chloride, 1.0%.

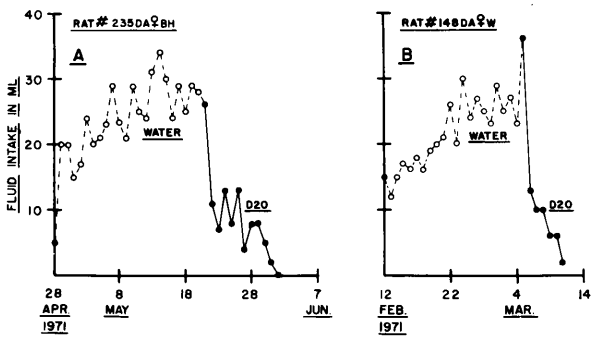


FIG. 1. Daily intake of distilled water for two rats; then restriction of intake to heavy water (99.8%).

the other. Then a subliminal concentration of a solution of the substance to be tested, if nutritive is placed in the bottle from which the rat had been drinking the smaller amounts, and if toxic in the bottle with the higher intake of distilled water. In either instance, concentrations of solution are increased by small steps at regular intervals (usually one day) until the intake levels definitely show that the rat has recognized presence of the test substance.

For all the many substances tested this technique gave clear-cut consistent results, with only narrow margins of individual differences.

For the present experiment (undertaken in 1970 at once after discovering that rats do not survive on heavy water) heavy water in a 5% concentration was placed in the bottle from which the rat had been drinking the larger amounts of distilled water. Then, every other day the concentrations were increased by 5% until they reached 99.8%.

Nine adult rats were used for this experiment. Records were made daily of intake from the two bottles; the rats were weighed every second day.

It must be stated here at once that the wide range of individual differences of reactions of the rats to heavy water make it impossible to give an average curve as was always possible with the previously tested substances.

Figure 2A shows the records for one of the rats that did not recognize a difference between heavy water and distilled water. Ordinates show daily intake in milliliters and body weight in grams; abscissas show time in days; the stepograph shows concentrations of heavy water from 5 to 99.8% (100). Intake of distilled water and heavy water fluctuated up and down right up to the end. On concentrations above 50% the rat began to lose weight showing that it was ingesting harmful amounts of heavy water. Interchanging positions of the bottles produced only a temporary decrease in intake of heavy water. Three other rats showed similar eratic records with no definite recognition of heavy water.

Figure 2B shows the record of a rat that drank only heavy water up to 80%; then after interchanging positions of bottles drank less heavy water for 3 days; then drank very large amounts and as a result died. It had lost weight at a rapid rate for the previous 7 days.

Figure 3A shows the record of a rat that after marked fluctuations in intake of distilled water and heavy water finally made a clear-cut recognition. It continued to gain weight throughout. Two other rats showed similar records. Figure 3B shows the only record that bears any resemblance to those obtained on the other substances. The rat recognized the heavy water solution in concentrations of 50% and above. It was then decided to give the rats a choice simply between heavy water (99.8%) and distilled water.

*Are rats able to recognize heavy water when given a choice of distilled water and a 99.8% concentration from the very start?* The technique was the same as before. The bottle from which the rat had been drinking the larger amounts was filled with heavy water (99.8%). Daily records of intake of distilled water and heavy water were then taken for several weeks.

The two bottles on each cage remained in

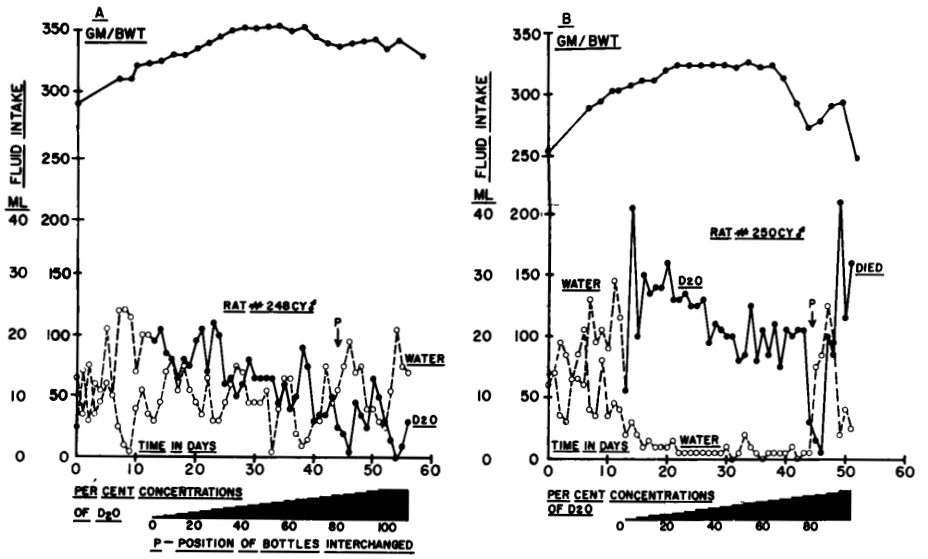


FIG. 2. Preference threshold records for two rats that had a choice of distilled water and heavy water in increasing concentrations (5% every other day) from 5 to 99.8%.

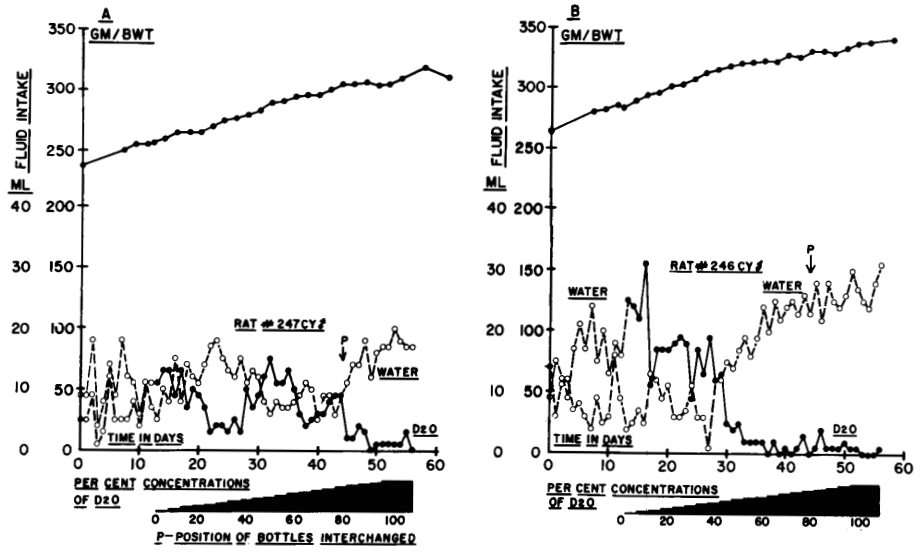


FIG. 3. Preference threshold records of two other rats.

the same position throughout the experiment unless otherwise specified.

Eighteen rats were used for this experiment.

*Results.* Figure 4A shows the record of one of these animals. On the first day this rat drank heavy water just as freely as it had been drinking distilled water. On the following days it practically stopped drinking heavy water but increased its distilled water intake to a normal level. After positions of the bottles were interchanged (P) the rat still refused to drink the heavy water. Here the rat definitely recognized the heavy water and almost showed an aversion to it. It gained weight at a constant rate throughout. Fourteen of the rats showed this type of record.

In view of the eratic unpredictable reactions of the nine rats in the "preference threshold" experiment, the fact that 14 out of 18 rats gave the Fig. 4A type of record

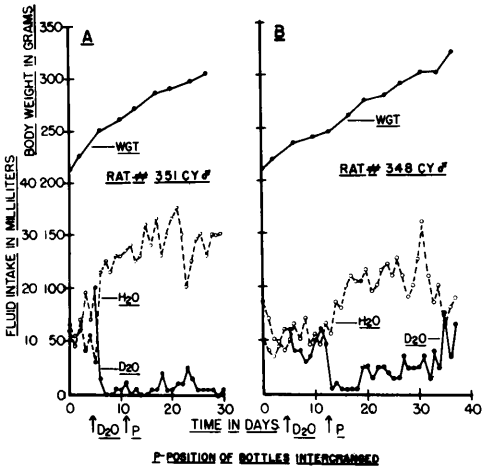


FIG. 4. Choice of distilled water and heavy water (99.8%) for two rats.

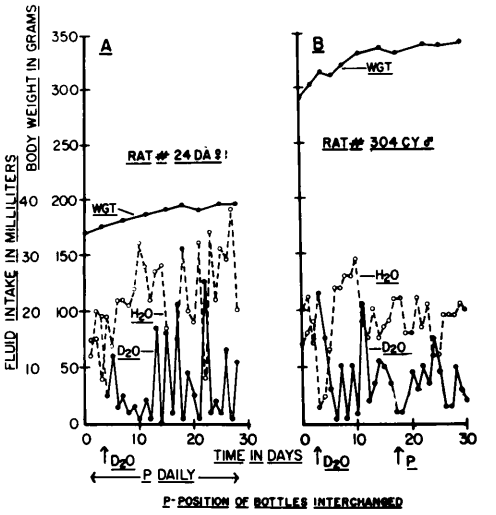


FIG. 5. Choice of distilled water and heavy water (99.8%) for two other rats.

must be of considerable significance.

Figure 4B shows a different type of record. This rat started by drinking almost equal amounts of distilled water from the two bottles. For the first 8 days it drank heavy water and distilled water in nearly equal amounts. After interchanging positions (P) of the bottles it stopped drinking heavy water for 6 days and took larger amounts of distilled water. Then it drank progressively more heavy water, finally drinking almost equal amounts of each. Body weight, however, increased at a steady rate throughout, indicating that so far the rat had not ingested harmful amounts. Two rats showed this type of record. These records and those in Figs. 5A and B are probably further instances of eratic behavior seen in the "preference threshold" tests.

Figures 5A and B show still two other types of records. In Fig. 5A, positions of the bottles were interchanged every other day. This rat apparently drank enough heavy water on the first 2 days to produce a temporary aversion. After that it failed to differentiate. Position habits apparently made it drink fairly large amounts of heavy water every other day. However, it did not ingest enough heavy water to cause more than a very slight decrease in weight gain. In Fig. 5B the rat drank almost equal amounts of distilled water and heavy water at the start; then drank smaller amounts of heavy water. Changing of positions of bottles (P) did not have any definite effect on the fluid intake.

Results of these experiments indicate that when on the first day the rats drank high amounts of heavy water and little or no distilled water, that they then developed a definite aversion to heavy water; whereas, when on the first few days they drank equal amounts of distilled water and heavy water, in some instances they did not make the distinction for 20–40 days or more.

This finding could mean that ingestion of high amounts of heavy water on the first day, that is when the rats drank it as freely as distilled water (i) they experienced untoward symptoms and (ii) they detected some characteristic of heavy water that made it possible for them to distinguish it from the distilled water and so to associate it with untoward symptoms.

What are the untoward symptoms? There is, of course, no way of determining what the rats experience. Apparently, they do not experience the untoward symptoms during the first 24 hr, since during this time they continued to drink heavy water as freely as distilled water. To throw light on this question determinations were made of heavy water content of the blood at various times after the start. Blood was taken at 8-hr intervals from the conjunctival sac in the corner of an eye with the method of Halpern and Pacaud (8).[3] Figure 6 shows curves

[3] Dr. Josef Pitha, Department of Gerontology of the

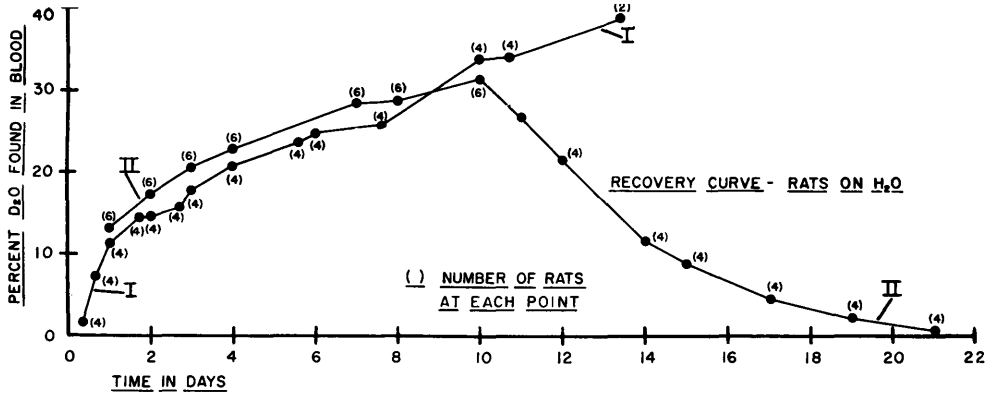


FIG. 6. Heavy water content of blood of rats whose fluid intake was restricted to heavy water (99.8%); and later to distilled water.

from two sets of readings. At the end of the first 8 hr heavy water content had reached about 7% and at end of the first day 12%, at the end of the second day 15–17%, and after 10 days as much as 30%. We have no idea what symptoms these amounts of heavy water produce. Previous experiments demonstrated that first signs of slowing of the 24 hr clock occurred on the second day and it is likely that many organs showed very slight slowing effects (2). Various symptoms together might suffice to produce untoward symptoms enough to elicit the aversion to heavy water.

The following experiments were undertaken to determine what characteristics of heavy water that the rats might associate with the untoward symptoms.

*Do rats taste heavy water?* This experiment was undertaken to determine whether the rats are able to taste heavy water. For this purpose taste nerves were cut in nine rats before the sharp differentiation shown in Fig. 4A was made; and in three rats sometimes afterwards.

The chorda tympani, glossopharyngeal, and the pharyngeal branches of the tenth nerves were cut or avulsed through exposures and methods described with Malone (9) in 1956. Taste buds in the fungiform, folliate, and circumvallate papillae degenerated after this operation.

*Results.* Figure 7A shows the record of one of the rats in which the nerves were cut before the differentiation was made. It shows the intake of distilled water for 20 days after sectioning of the nerves. Heavy water was then placed in the bottle from which the rat had been drinking the larger amounts. On the first day, the rat drank heavy water as freely as distilled water. On the next day, however, it almost completely stopped drinking it, thus, demonstrating that the differentiation did not depend on the ability of rats to taste heavy water. The other eight rats showed the same results. Figure 7B shows that sectioning of the taste nerves after a clear cut differentiation had been made did not interfere with the rat's ability to recognize the heavy water. Interchanging positions of the bottles as well as interchanging of distilled water and heavy water without changing positions of the bottles did not alter the results. The other two rats gave the same results.

*Are rats able to smell heavy water?* This experiment was undertaken to determine whether rats are able to smell heavy water. For this purpose olfactory bulbs were removed in 16 rats before the differentiation was made between distilled water and heavy water, and were removed in six rats afterwards.

*Results.* Figure 8A shows the record of one of these rats. The olfactory bulbs were removed 8 days before start of the heavy water which was placed in the bottle from which the rat had been drinking the larger amounts of distilled water. This rat drank heavy water as freely as distilled water, not only on the first day, but on the 14 following days right up to the time of its death. It did

National Institute of Health in Baltimore and Dr. Catherine Fenselau have my gratitude for many helpful suggestions and use of the spectrophometer.

not, at any time, start to drink more distilled water. This record shows that this anosmic rat could no longer recognize the difference between heavy water and distilled water. Body weight showed a sharp decrease from the start with heavy water. Ten other anosmic rats showed this same result.

Figure 8B shows the record of an anosmic rat that continued to drink fairly large amounts of distilled water along with large amounts of heavy water. It lost some weight but did not die until 35 days after start of the heavy water. By that time its body weight had dropped far below the predicted level. It clearly did not differentiate between distilled water and heavy water. Figure 8C

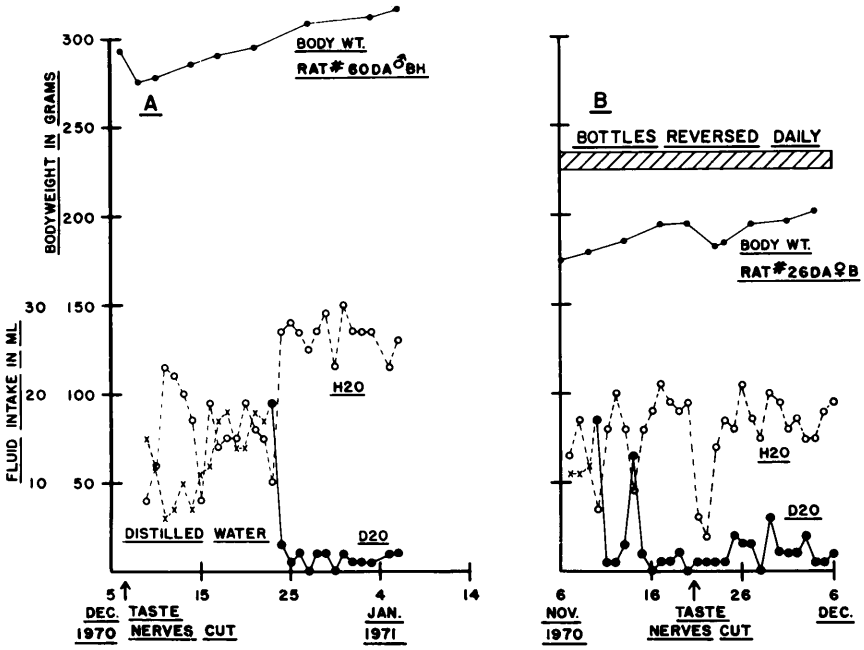


FIG. 7. Graphs showing that rats still recognize heavy water after sectioning of taste nerves: (A) before choice; (B) after choice.

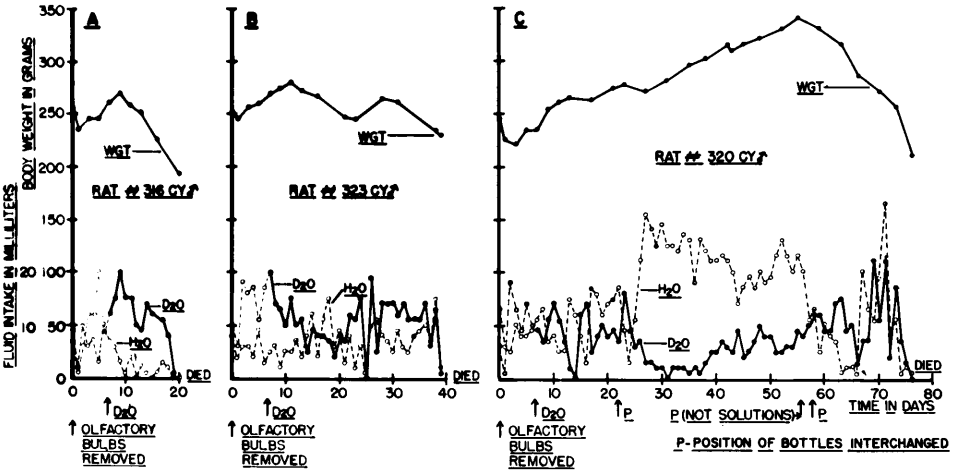


FIG. 8. Three graphs showing inability of rat to recognize heavy water (99.8%) after removal of olfactory bulbs.

shows a record of another anosmic rat which did not die until 73 days after the start of heavy water. For the first 20 days it drank slightly more distilled water than heavy water; then, over a long period of eratic behavior it finally drank heavy water in lethal amounts. Six rats showed the types of curves shown in Figs. 8B and C. They all finally drank lethal amounts of heavy water. Of the six rats from which the olfactory bulbs were removed after the differentiation had been made three failed to differentiate and died after 13, 28, and 29 days; but three rats still differentiated. These last three anosmic rats must have still detected the presence of the heavy water in spite of changes in positions of the bottles for fluids, use of entirely new sets of bottles, and reoperations to make certain of removal of all olfactory tissue.

*Are rats able to detect the difference in viscosity between distilled water and heavy water?* At room temperature heavy water is 15% more viscous than distilled water; at 5° it is 31% more viscous (2). Reactions to differences in viscosity might be detected by tactile sensations (lingual nerves).

One differentiation experiment was conducted on nine rats in a cold room (5°). Another experiment was conducted on six rats at room temperature after sectioning of the lingual nerves and presumably eliminating tactile sensations.

Neither experiment gave definite results. Viscosity could not play an important part since at 5° the rats failed to differentiate, due in part, also, to reduced aerosal in the cold. Thus, the ability of the three anosmic rats to differentiate between heavy water and distilled water still remains unexplained.

*Discussion.* Eratic variability of intake under similar conditions by rats puts heavy water in a category different from any other substances that have been tested. Some rats started to drink heavy water again after having clearly shown a strong aversion for some time. They did not, however, ingest lethal amounts, except in one instance.

Rats avoid heavy water after a combination of two circumstances: (i) intake of high amounts of heavy water, high enough to produce definite untoward symptoms after about 24 hr on first exposure and (ii) exposure of the rat to enough heavy water aerosal to make an association between untoward symptoms and the smell of heavy water.

Thus, with the three exceptions, anosmic rats gave the same type of curves for heavy water that were obtained earlier (6) with the highly soluble and toxic chemicals sodium fluoroacetate (1080) and thallium sulfate. These two chemicals have no taste and no smell to either rats or human beings. This makes them such effective poisons. The rats drank them freely and were not able to distinguish them from distilled water. In the same way, anosmic rats drank heavy water freely and were unable to differentiate it from distilled water.

That 1080 and thallium sulfate have no taste to rats or man and probably to all other animals, was explained by the fact that animals have never been exposed to these chemicals in nature and so, in the process of evolution, have never built up an ability to protect themselves against them. Likewise, heavy water has never been present in nature except in the most minute amounts and it is only very slightly toxic anyway, so animals have never evolved an ability to taste it. That they are able to smell it is purely coincidental.

*Summary.* Restricted to heavy water (99.8%), rats drank it freely for the first day, then they drank progressively less and died within 14 days. When given a choice between distilled water and heavy water (99.8%) rats avoided heavy water, partly by virtue of some deleterious effects of heavy water and partly by virtue of a faint smell of heavy water with which the untoward effects could be associated. Rats did not taste heavy water. Whether a higher viscosity of heavy water plays any part could not be determined. Apparently the low intensity of any untoward effects of heavy water and the faintness of its smell could explain the eratic variability of the reactions of rats to heavy water that put it in a different category from other substances tested so far.

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