THE BASIS OF THE PHARMACOLOGICAL ACTION OF HEAVY WATER IN MAMMALS*

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Among all isotopes those which would be expected to show the greatest differences from the original element are the isotopes of hydrogen. The addition of one or two protons to a relatively large atom is an insignificant change in comparison with that undergone by the transformation of hydrogen to its nearest isotope, deuterium, for here the extra proton practically doubles the mass. In fact deuterium is frequently described as "like a new element."

The following are some of the changes imparted by deuterium to water when it replaces hydrogen to form deuterium oxide, or heavy water. The melting point and boiling point are increased respectively by 3.82° C. and 1.42° C. The solubility of sodium chloride is decreased by 15 per cent, viscosity is increased by about 25 per cent, the vapor pressure is lowered, and specific gravity is increased to 1.1074. A number of these properties would certainly be expected to lead to changes in living organisms where considerable proportions of the body water are replaced by heavy water. In fact, when Urey[41] first made deuterium oxide available, they predicted that it would prove of considerable biological interest.

When appreciable amounts of deuterium oxide enter the animal organism problems immediately arise which concern the capacity of the tissues to exchange hydrogen for deuterium. Chemists have already given us information as to the stability of hydrogen variously placed in organic compounds. The following table by Bonhoeffer[9] indicates how readily exchange of hydrogen occurs between organic compounds and H_2O. It was found by tracing the exchange of deuterium atoms:

Benzol, pyridin, Na acetate	: none
Cane sugar, glucose	: OH groups only
CH_3CHO	: slow (one H)
HCHO	: slow (H atoms)

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Acetone	neutral	: slow
	acid	: all H atoms
	alkaline	: all H atoms rapid
Protein		: all N-bound H atoms (30%)
Cellulose (several days at 100°)		: all OH groups

Briefly stated, it is known that about one-third of the hydrogen atoms in proteins and more in carbohydrates interchange freely with deuterium atoms which may be present; but fats are lacking in labile hydrogen. The last mentioned fact has made it possible for Schoenheimer[35] to contribute fundamentally to our knowledge of fat metabolism by the expedient of tracing in the body the fate of fats "marked" by the possession of stable deuterium atoms.

Deuterium oxide is found everywhere as a natural component of ordinary water, the generally accepted concentration being in the neighborhood of one part in 5,000 (.02 per cent). Despite numerous claims to the contrary there is little evidence of variation of this proportion in living organisms or the water obtained from various mammalian organs and tissue fluids including those of quite old men and oxen, including also such important foodstuffs as milk and orange juice. All contain approximately the same proportion of heavy water.

Absorption and Fate of Heavy Water in Animals. The deuterium oxide molecule has a radius only 0.1 per cent greater than that of H_2O, whence it follows that diffusion of the two kinds of water occurs at essentially the same rate under the same conditions. That the heavy water passes readily through various types of animal membranes was early shown by v. Hevesy and Hofer[21] in experiments on goldfish. The fish were placed in water containing known concentrations of deuterium oxide and were killed at various intervals for determination of the composition of the body water. Equilibrium was complete within four hours, and the reverse process, obtained by placing the deuterium-treated fish in ordinary H_2O, proceeded with equal rapidity.

The frog skin has been made the subject of special studies by v. Hevesy, Hofer, and Krogh,[22] who, by using deuterium as a tracer, were able to demonstrate that this tissue has the same permeability for water in both directions (settling a much disputed question), and that it is a thousand times less permeable to water than is a collodion membrane. They showed further that the permeability of frog skin to water was lessened by cooling but could not be influenced by severance of its connections with the central nervous system.

In rats and mice small amounts of deuterium oxide become equally distributed throughout the body water within an hour. This was demonstrated by McDougall, Verzar, Erlenmeyer, and Gaertner.[29] They injected into jejunal loops isotonic xylose in 1.64 per cent D_2O. After one hour the concentration of D_2O present in the loops corresponded with the dilution which would have been obtained from mixing the original deuterium with an amount of water equal to two-thirds of the body weight.

Thus, it became clear that heavy water is readily absorbed from the alimentary tract and passes through a variety of animal membranes at somewhat the same rate as does ordinary water. More exact determinations by Lucké and Harvey[26] on sea-urchin eggs revealed no difference between the rate of entrance of H_2O and D_2O, but in the case of red blood cells both Parpart[32] and Brooks[12] have found the entrance of D_2O decidedly slower than that of H_2O. von Hevesy and Hofer,[20] as a result of experiments on themselves, concluded that this facility of penetration of heavy water made it possible to determine the average duration in the human body of an ingested molecule of water, as well as to estimate the total amount of water in the body. They each drank two liters of 0.46 per cent D_2O. Deuterium first appeared in the urine in 26 minutes, indicating (by the known sensitivity of their method) that 1 cc. of the two liters was already excreted. One-half the deuterium oxide, presumably one-half the ingested water, was excreted within ten days. The average stay of a molecule was calculated as $\frac{10}{\ln 2} = 14$ days. This shows that most of the water taken becomes completely mixed with the water content in the body. They calculated from their data, that the body water amounts to 63 $\pm$4 per cent. The superior sensitivity of deuterium as an indicator in the body may be judged from the fact that while the ingested material appears in the urine in 25 minutes and does not disappear for weeks methylene blue requires about two hours for appearance and disappears in 43 hours.

Further evidence of the complete mixture of D_2O with the body water has been obtained by Barbour and Trace[5] in mice. The insensibly lost water of many of these animals kept on long courses of heavy water was collected in 100 cc. pipettes packed in solid carbon dioxide; 0.01 cc. suffices for a determination by the falling-drop method of Barbour and Hamilton.[2] In the last two columns of Table I will be seen the agreement between the specific gravity determinations of the final samples of insensibly lost water and those of the water distilled from the whole carcass. These data show that elimination through the lungs and skin of D_2O and H_2O is sufficiently uniform to make the insensibly lost water an active index of the body water.

An example of the permeability of the placenta for heavy water is seen in the following density data: Insensibly lost water of a parturient female mouse: 1.0139; of the combined body water of two young a few hours after birth on the same day: 1.0146 (Barbour and Trace[5]).

In living organisms it has been abundantly shown that the labile hydrogens will always exchange for deuterium proportionally to its percentage in the surrounding fluid. For example, P. K. Smith, Trace, and Barbour[36] showed that the oxidation water from the tissues of mice killed one or more hours after receiving single injections of heavy water in various amounts contains D_2O to the extent of about 18 per cent of the D_2O percentage in the body water. It will be recalled that about 30 per cent would be expected in equilibrium with proteins, more with carbohydrates, and none in equilibrium with fats. Ether-soluble tissue was found, however, to contain some labile hydrogen, in fact, about one-third as much as the remaining nitrogen-rich fraction.

But in growing organisms an additional phenomenon is seen, namely, the incorporation of deuterium into stable positions. This was discovered by Reitz and Bonhoeffer.[34] They found slow assimilation of deuterium in chlorophyllic organisms grown in 12 per cent D_2O. The deuterium content of dried algae, at the end of an experiment, was 5.2 per cent, of which about one-third was bound directly to carbon. Growing yeast did not show this phenomenon. In our own experiments (Barbour and Trace[5]) mice drinking 15 per cent deuterium oxide for two months showed three times the concentration of deuterium (relative to body water deuterium) in the tissues as did mice receiving a single injection of heavy water. This is evidence for the fixation of deuterium in living mammalian tissues in a stable form. Krogh[25] reports substantial fixation of deuterium in mammalian muscle, liver, and bone.

All evidence therefore seems to indicate that when a living organism ingests D_2O there is an immediate proportional exchange of D with the H of hydroxyls and amines, while a very slow binding of D with C commences.

Enzymes

Certain enzyme reactions are known to be catalysed by hydrogen ions, among these the muta-rotation of alpha-d-glucose. This reaction, according to Pacsu,[31] occurs three times as rapidly in H_2O as in concentrated D_2O, the difference being greatest at the lowest temperatures. Steacie[37] found that the hydrolysis of cane sugar at 45° C. proceeds 1.4 times as rapidly in H_2O as in D_2O and 1.8 times as rapidly at 18° C. Barnes and Larson[6] reported that .06 per cent D_2O retards somewhat the digestion of starch by pancreatic amylase and the fermentation of glucose by zymin. Macht and Bryan[27] found a considerable increase in the velocity of reaction of oxidases from muscle and brain when D_2O was present in concentrations of 1:100 or 1:2000. A bean catalase was apparently accelerated by more than 50 per cent in 1 per cent deuterium. In studying a number of enzyme reactions similar to those mentioned above, Fox[14] obtained negative results, both with one per cent and 99 per cent heavy water. One feels, however, on much safer ground when discussing the effects of the higher percentages of deuterium oxide. Alcoholic fermentation of glucose in H_2O, for example, has been found to proceed nine times as rapidly as in 100 per cent D_2O and 1.6 times as rapidly as in 60 per cent D_2O (Pacsu[31]). Harvey and Taylor[19] found the respiration of a salt-water luminous bacterium reduced 60 per cent by 86 per cent heavy water; luminescence was also markedly diminished. The respiratory reduction in 36 per cent heavy water was 12 per cent. Less striking, but similar, results were seen with a fresh-water bacterium. The same authors studied the respiration of yeast, with the result that the oxygen consumption was found decreased by D_2O concentrations of 20 per cent or more. A 50 per cent depression was produced by 97 per cent D_2O.

It is with concentrations in the body water of from 20 to 50 per cent that satisfactory knowledge of the effects of heavy water on mammals is now accumulating.

Bonhoeffer and Salzer[10] have recently discussed the superficially contradictory results of deuterium oxide in a single group of reactions, the hydrolysis of glucosides. They find that some glucosides, for example, salicin, as found by Steacie,[38] are split slower in heavy water than in light water while others, of the methyl glucoside type, are split more rapidly. The accelerating effect is ascribed to an increased affinity between enzyme (emulsin) and substrate which overcompensates for the delayed hydrolysis. To account for this the heavy water is said to delay the breakdown of the enzyme-substrate complex; thus a depressing effect of D_2O would indirectly accelerate hydrolysis. Harvey's[18] viewpoint that deuterium affords a "generally unfavorable environment" for biological activity and exerts "differential effects on the rate of biochemical reactions, which ordinarily proceed at a certain definite rate in relation to each other" appears to harmonize the multitude of biological effects of heavy water thus far ascertained. At bottom lies the fundamental fact emphasized by Barnes that systems containing deuterium exhibit a low energy content.

Some Effects of Deuterium on Complex Animal Systems

Before systematic studies were begun on mammals in this laboratory further evidence supporting the general view of deuterium as a depressant had accumulated. Taylor, Swingle, Eyring and Frost[40] first established the lethality of deuterium oxide for some of the higher animals, finding that tadpoles died in fifteen minutes in 92 per cent D_2O while surviving three days in 30 per cent D_2O. Also, a common aquarium fish was killed within two hours, and flat-worms within four hours, by the stronger concentration. (Paramecia survived two or three days.) Most of these forms appeared unharmed by a 24-hour exposure to 30 per cent D_2O.

Depression of the heart by deuterium oxide was first described by Barnes and Warren,[8] who noted a decrease in the rate of pulsation of the excised heart of the frog. This was later confirmed by Brandt and Reindell,[11] who described electrocardiographic changes. Verzar and Haffter[43] have also noted inhibition of the rate and amplitude, as well as decreased irritability.

Barnes and Warren[8] studied exhaustively the effects of high concentrations of deuterium oxide upon isolated auricles of the turtle heart. They describe the effect of heavy water (approximately 75 per cent) as "equivalent to a drop in temperature of about 5° C." The amplitude of the auricular beat was lessened and standstill occurred much sooner than in the controls. A linear relation was shown between the logarithm of the rate of pulsation and the reciprocal of the absolute temperature. Although the temperature characteristics showed occasional variations this was not considered evidence that heavy water had necessarily changed the master reaction. The slowing of the beat was regarded as largely a matter of reduction in energy, because of the striking recovery on return to ordinary water. Barnes believes that the unique properties of heavy water make it possible to investigate the effects of reduced energy without change in temperature.

The Effects of Deuterium Oxide on Mammals

The results on mammals, about to be described, seem to offer no obstacles to the concept that fundamentally deuterium is a depressant of biological activity. Like those of most pharmacological agents its effects can be reversible or not depending upon the concentration. The fact that many evidences of stimulation are found may serve merely to illustrate the complexity of the systems affected. The work in the Yale Pharmacological Laboratory has to date been done chiefly on female mice between the ages of 80 and 120 days from the inbred stock of the Anatomical Laboratory. Most of them have been used in metabolism work for which purpose they were ovariectomized, to eliminate variations due to the sex cycle.

If a mouse receives pure deuterium oxide subcutaneously or by mouth daily, in three doses amounting to 1 cc. per 10 grams per day, there appears after about 24 hours a general pilomotor effect (Barbour and Trace[5]). The back hair is erected, the collar ruffled, and the belly appears pink. There is also an unusual persistence of the exophthalmos resulting when any excitement occurs. As the animal progresses from about 10 per cent to 20 per cent saturation, as indicated by the specific gravity of the body water, an increase in the total metabolism is noted, which, at the latter level, averages nearly 20 per cent above normal (Barbour & Rice[4]). If the deuterium oxide be continued, but its concentration now reduced to 40 per cent instead of 99.5 per cent, the metabolism is maintained at the new level for many days, slowly settling to the normal level some days after the heavy water has been stopped. A thyroidectomy which reduced a mouse's metabolism by about one-third had no effect upon the subsequent calorigenic effect of heavy water other than to make it appear greater. With the metabolism increase is associated a rise in the body temperature which may be held nearly 1° C. above the normal level for several days.

With concentrated heavy water a crisis is reached at about the fifth day when the body water has become about one-third saturated

TABLE I

LETHAL EXPERIMENTS WITH DEUTERIUM OXIDE IN FOURTEEN MICE

D_2O given			*Water Supply**			*Specific Gravity*	
Concentration	*Route*	*Total to death*	*Preliminary H_2O*	*D_2O*	*Body Weight Change*	*Insensible Water Loss (Final)*	*Body Water (Carcass)*
		cc./10gms. of mouse			*gms.*	*(Sp. Gr.-1)x* 10,000	
99%	Subcutaneous	6.0	E	E	+1.9	540	561
		5.6	E	E	—0.9	471	474
		5.25	E	I	—3.5	451	441
		6.0	I	E	+5.4	481	
		7.2	A	A	—1.9	450	
	Stomach Tube	5.2	E	E	+0.9	472	480
		7.3	E	A	—3.3	485	
	Fountain	5.8	A	I	—8.0	317	331
		2.0	A	I	—7.0	121	097
50% (Equal parts of H_2O added)	Subcutaneous	8.0	E	E	+1.3	406	409
		4.5	I	E	—1.2	321	298
	Stomach Tube	5.2	A	A	—6.6	291	
		6.5	A	A	—4.0	340	335

* E = Excessive: 1.5-2.0 cc./10 gms./day.
A = Adequate: 1.0-1.4 cc./10 gms./day.
I = Inadequate: 0.4-0.8 cc./10 gms./day.

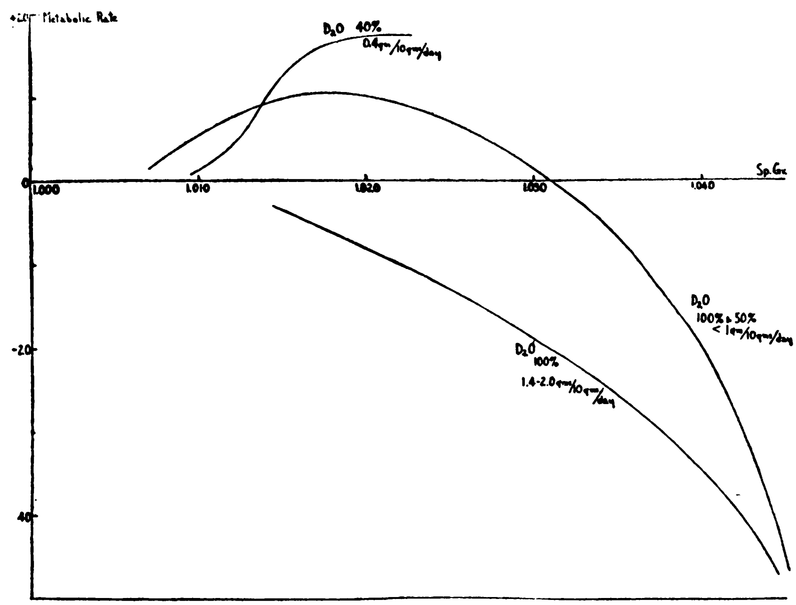
with deuterium oxide. Body temperature and metabolism begin to fall and definite hyperexcitability to external stimuli is noted. About this time the animal will give spontaneous leaps in the chamber or, if removed, will exhibit violent jumping reflexes when touched. Sometimes a noise or merely a blast of air will initiate such a jump. Such extreme irritability is reminiscent of strychnine poisoning, but no convulsions of any sort occur. From this time on, the temperature and metabolism fall steadily and the mouse becomes somewhat cyanotic and ataxic. Toward the end it exhibits dyspnea and general depression but may survive many hours at a metabolic level only 20 per cent of the normal. Death occurs at or near the seventh day, the body temperature, if in the usual 28° C. environment, having fallen to 30° C. or below (Barbour and Trace[5]).

When pure heavy water is given at the above rate, which corresponds to an adequate normal water supply, from 5 to 7 cc. per 10 grams of mouse are necessary to produce death (Table I). Similar amounts are lethal when given in 50 per cent solution, that is, when equal parts of H_2O are added to the D_2O dose. The latter experiments show that the chief toxic effects seen after heavy water are not due to H_2O lack. "Water intoxication" is of course excluded as a cause of death in all experiments where the total water given was not excessive. In the table are cited experiments in which, during the preliminary period of four or more days, the H_2O intake has been either "excessive," "adequate," or "inadequate"; conditions were similarly varied during the D_2O period. The principal effects of thus changing the water levels are reflected in the body weight changes. The body weight under treatment with concentrated heavy water is only maintained at the normal level by giving excess water; it falls definitely during concentrated deuterium oxide administration if the water is merely "adequate." In the two experiments where mice received their heavy water only from a fountain, death occurred at unusually low deuterium levels because, after the first day, the mice would not drink adequate amounts voluntarily.

The specific gravity data show that nearly all the mice approach half saturation with D_2O before death except where inadequate water ingestion complicates the picture. It has been possible in most cases to calculate approximately the complete water balance during administration of heavy water. When uncomplicated by any change in water dosage at the time the D_2O is begun these water balances have shown some retention by the body on the first day or two of heavy water administration. This is confirmed by the upward turn of the body weight curve uniformly seen at that time. The retention is sometimes associated with oliguria.

The relation of water loss to metabolism has been carefully investigated during the period of rising metabolism. There is at this time constantly a lag in the insensible water loss increase; in other words, the $\frac{H_2O}{CO_2}$ ratio is significantly decreased. This is a phenomenon which has been noted (Marshall, Aydelotte, and Barbour[28]) in acute experimental fevers, and is probably intimately associated with the rises in temperature and metabolism noted.

The metabolic effects of three different types of heavy water administration are illustrated in Figure 1. Metabolism changes (ordinates) are plotted against levels of saturation with deuterium



Effects of deuterium oxide on total metabolism in mice in relation to deuterium saturation of the body water. Ordinates, changes in metabolic rate; abscissae, specific gravity of the insensibly lost water.

as indicated by the specific gravity of the body water (abscissae). One-fifth saturation corresponds, for example, to a specific gravity of 1.0215. The upper curve (46 determinations) shows the stimulation in metabolism when mice are brought up to the level of one-fifth saturation, either rapidly, as by two days of concentrated heavy water, or slowly, as by nearly a week of 40 per cent dosage. Owing to the H_2O content of the food and the inevitable loss of deuterium to the air the 40 per cent dosage is required to assure one-fifth saturation. The middle curve (42 determinations) shows the stimulation of the metabolism followed by depression when the mice are given 100 per cent or 50 per cent heavy water at a rate of less than one gram per 10 grams per day. The lowest curve (10 determinations) shows the effect on the metabolism of giving larger doses of concentrated heavy water.

The metabolism fall (shown in the middle curve) during the last three days of life was preceded slightly by a temperature fall, to which it may have been due. In view of this and of the onset of profound central nervous system disturbance it was suspected that the metabolism fall was entirely secondary to other functional changes. To test this hypothesis numerous metabolism experiments have been performed in an environment of 32° C. instead of the usual 28° C. Here it was found that the central nervous changes and death occurred at the same time as, or slightly earlier than, at the lower temperature, while the fall in both body temperature and metabolism were delayed until the last day. This shows that death from heavy water is not due primarily to depression of the body temperature or metabolism.

Hansen and Rustung[17] have reported that deuterium oxide failed to modify metabolism in mice; but, in their experiments the heavy water was given in small doses mixed with the food, and the variations shown by the controls were great, the animals apparently not having been castrated.

Effects of Heavy Water on the Sympathetic Nervous System

The early pilomotor effect of deuterium oxide has been examined by Barbour and Herrmann[3] with a view to its possible connection with the sympathetic nervous system. Twenty-gram mice were given doses of about 1 cc. of heavy water at intervals of from one-half to two hours. After about 4 cc. had been given, and the animals were approximately one-fifth saturated, the pilomotor effect and exophthalmos were usually marked and persistent. It was found that these effects could be completely removed within one hour or less by the injection of less than one milligram of ergotoxine ethanesulfonate. This strongly indicated a sympathomimetic influence of heavy water.

Experiments on the melanophores of isolated fish scales by Barbour and Bogdanovitch[1] lend support to the sympathetic theory. Such melanophores are well known to exhibit contraction on exposure to very weak solutions of epinephrine, a contraction which ergotoxine not only prevents but converts into relaxation. Sufficiently ergotized melanophores will no longer contract under the influence of deuterium oxide up to 25 per cent. Furthermore, potentiation betweeen epinephrine and deuterium oxide is demonstrable in melanophores.

In view of all the above evidence that heavy water is favorable to the activity of the sympathetic nervous system, its calorigenic action was examined in four mice with the uniform result that the metabolic stimulation induced by heavy water could be held in abeyance by the administration of ergotoxine ethanesulfonate, 0.3 milligram per 10 grams of mouse, just previous to the six-hour metabolism run (Barbour and Rice[4]).

All these effects of facilitation of the sympathetic nervous system occur clearly when the body water is about one-fifth saturated with deuterium oxide. This is a concentration to which inhibition of some enzyme reaction could definitely be ascribed. The hypothesis is therefore advanced that heavy water tends to protect the sympathetic hormones which may be present in the body by interfering with the normal removal, by oxidation, decomposition, or otherwise, of their influence.

On this hypothesis one would expect that actions other than those on the sympathetic nervous system might be enhanced by deuterium. This, in fact, from preliminary experiments by Barbour and Bogdanovitch,[1] appears to be the case in melanophores treated with acetyl-choline.

Looking still further, to the central nervous system, Barbour and Herrmann[3] have found the convulsive action of ergotoxine facilitated by the presence of large amounts of heavy water. A mouse about one-fifth saturated with deuterium oxide will succumb to less than one-half the minimal lethal dose of ergotoxine. We have found further a persistent effect from introducing small amounts of heavy water into the cerebrospinal fluid. This has been done by placing a few drops of deuterium oxide in the parietal region in seven rats, and by the method of lumbar puncture in two cats. All of these animals exhibited a marked degree of catatonia. Apparently the percentage of heavy water thus brought into contact with the brain tissue is identical with that to which the central nervous system would be exposed if the entire body water were 20 per cent saturated with deuterium oxide. Thus, there has been accomplished by the local action of a few drops of heavy water a depression of the central nervous system otherwise effected only by the use of about 200 cc. per kilo, a dosage at present prohibitive except for mice. When such an amount is given over a short space of time the picture is complicated by signs of "water intoxication," such as rolling movements and muscular twitching, as Hansen and Rustung[16] have also observed.

Can Deuterium Oxide Become Useful in Medicine?

At this date it seems premature, as has beeen attempted in some quarters, to predict therapeutic uses for deuterium oxide. To show its relative harmlessness in amounts producing many pharmacological actions, a mouse now drinking heavy water may be mentioned. This animal, while for over two months limited in its water intake to the voluntary ingestion of forty per cent deuterium oxide, has shown retarded gain in weight but no other apparent ill effects. Other mice have been desaturated after one-fifth saturation without noticeable harm.

Uses in the field of antisepsis have doubtless been in the minds of those who have made bacteriological tests with heavy water. Most of these appear to be quite negative, for example, Hansen[15] found that 92 per cent deuterium oxide over a period of 40 to 70 days exerts neither inhibitory nor lethal effects on *Staphylococcus albus* or typhoid bacilli. Dujarric de la Riviere and Roux[13] have also reported negative results with a dozen of the common pathogenic microorganisms. Eighteen per cent deuterium oxide yielded negative results on the growth of staphylococcus and on the motility of *B. typhosus*, in the hands of Kredel and Harkins.[24] Increased growth of the tubercle bacillus has been described by Itoh, Inoshita, and Titani,[23] who used 2.24 per cent deuterium oxide.

In the field of malignant tumors it will probably be a long time before a final verdict can be given. Sugiura and Chesley[39] have been unable to influence mouse sarcoma and rat carcinoma by 24-hour immersion of such tumors in 14, 40, or 94 per cent heavy water. Negative results for sarcoma have also been obtained *in vivo* by Woglom and Weber.[44] Rea and Yuster[33] found in 44 of 50 rats that 0.11 per cent D_2O added to tumor inoculations or injected around the tumor (rat sarcoma) exerted no influence. In six of the rats the tumor was apparently stimulated by the deuterium oxide. Other investigations bearing on this subject, including the effect of deuterium on growth in lower forms of life, have been discussed by S. L. Meyer.[30] The observations by Ussing[42] on the retarding influence of heavy water on the development of amphibian eggs are particularly inviting to a wider exploration of diseases in which cell growth and sulfhydryl mechanisms are vitally concerned.

Our investigations in mammals suggest speculation along many possible lines of therapeutic use. Heavy water is, however, a very scarce material and, judging from mice, it would today require a daily outlay of fifty dollars per kilo of body weight to maintain the interesting but perhaps harmful level of one-fifth saturation.

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