values deviate by more than 2 per cent from those which may be obtained from equation (2), and in each case the tendency is for the equilibrium to be shifted toward 0.5. The discrepancy is most marked where there is a large difference between S_1 and S_3 and where S_2 is small. The unstable equilibria are affected to a slightly greater extent (up to 5%) and are moved away from 0.5. In no case is the stability different from that obtained from (3).

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[1] Haldane, J. B. S., "Selection against heterozygotes in man," *Ann. Eugenics*, 2, 333–340 (1942).

[2] Gantmacher, F. R., *Applications of the Theory of Matrices* (New York: Interscience Publishers, Inc., 1959).

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*THE PATHOLOGIC ANATOMY OF DEUTERIUM INTOXICATION**

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In 1933, when Urey first described the separation and properties of deuterium,[1] he suggested that a "marked effect upon living organisms" might be produced in view of the physicochemical differences that existed between hydrogen and its heavier isotope. Shortly thereafter, Lewis[2] observed that tobacco seeds did not germinate in heavy water and did so only slowly in 50 per cent D_2O. This observation was the first in a long series of experiments that over the course of the ensuing thirty years has demonstrated the widespread and frequently unique effects on biological systems of deuterium oxide.

The purpose of this communication is to present the results of our investigations into the nature of the anatomic changes attendant upon the replacement of one third or more of the total body water of a mouse with heavy water. Considerable information has accumulated during the past three decades on the pathophysiology of deuterium intoxication,[3] however, very little information exists on the morphological effects observed in association with the extensive and diverse functional derangements that have been described.

Materials and Methods.—Two series of experiments were performed using 50% and 75% D_2O, respectively. Young male mice of the Swiss-Webster strain were offered water and food *ad libitum*. Experimental animals received 5% dextrose solution of the appropriate D_2O concentration; litter-mate controls were given 5% dextrose solution. Dextrose was added to the drinking water to induce high levels of water intake. In Experiment I, five experimental animals were sacrificed (chloroform) after 5 days on 50 per cent heavy water; six controls were sacrificed in a like manner one day later. In Experiment II, six animals received 75% D_2O for 6–10 days. Death occurred spontaneously in all these cases and autopsies were performed within 1 hr of death except

in one case (Experiment II, animal D 1). Three litter-mate controls were sacrificed at intervals ranging from 9 to 20 days. Observations were limited to the quantity of food and water consumed, changes in weight, and the behavior of the animals. At necropsy the following organs were weighed and representative sections taken for microscopic studies: heart, lung, spleen, liver, pancreas, adrenals, kidneys, testes, and thymus. In addition, sections of the following organs and tissues were taken for microscopic study: stomach, large and small intestine, urinary bladder, seminal vesicles, thyroid, salivary glands, brain, pituitary, skin, muscle, bone, and fat. All tissues were fixed in Bouin's solution and stained with haematoxylin and eosin. Analysis of D_2O concentration of samples of body water at time of death were obtained from liver tissues by vacuum distillation. Five-mg aliquots of these samples were converted to hydrogen by reaction with heated uranium metal and the gas analyzed for deuterium with a mass spectrometer.[4]

Results.—Clinical observations: Experiment I (50% heavy water): The first detectable change noted in the experimental animals was the development of generalized piloerection 24 hr after the commencement of deuterium ingestion. By the next day (48 hr) the deuterated animals were seen to be fighting with each other. Frequently unprovoked attacks, occurring in an apparently random manner, were observed. Generalized hyperactivity, combativeness, and piloerection were noted in the experimental group of animals for the duration of the experiment, at which time (5 days) they were sacrificed. The control animals were at all times observed to be normal in behavior toward each other and in general activity. It was observed that throughout the course of the experiment the fluid intake of the experimental animals was below that of the controls, with the former averaging 3.7 cc/animal/24 hr compared to 7.4 cc/animal/24 hr for the latter group. The deuterated animals also manifested a decreased intake of food throughout the duration of the experiment.

Experiment II (75% heavy water): The changes observed in the experimental animals were initially quite similar to those observed in Experiment I. However, hyperactivity was considerably more prominent. For the first several days the deuterated mice were in constant activity. Their appetites were voracious, and constant savage attacks upon each other were continually in progress. Often, when stimulated by another animal or one of us, they would withdraw in a series of what appeared to be rapid tetanic spasms. Occasionally, convulsive episodes were observed in response to external stimulation. Eventually, at varying intervals, the animals became hypoactive, lethargic, and dramatically weak. They would lie in a corner of the cage making no attempt to feed or drink or to resist attacks from their cage-mates who had not yet become as moribund as they were. The animals invariably exhibited a two- to threefold increase in respiratory rate. Terminal convulsions were frequently observed. Death occurred in all cases within 6–10 days. The concentration of heavy water in samples of body water obtained at autopsy ranged from 21.2 per cent to 27.0 per cent in animals D 2 to D 6. Animal D 1, who was the first to die and in whom autopsy findings included confluent bronchopneumonia, had a heavy water concentration of 37.4 per cent.

Autopsy findings: Experiment I: Gross and microscopic examination failed to disclose any anatomic alterations in deuterated or control animals.

Experiment II: Gross examination: Animal D 1 was found to have extensive con-

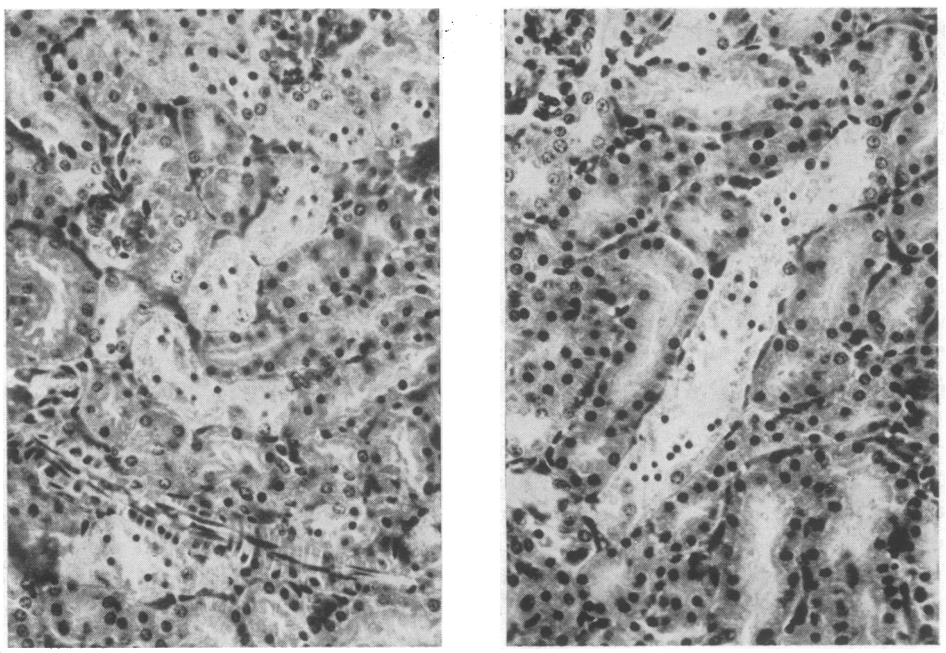

FIG. 1.—Tubular degeneration in the mouse kidney. The proximal convoluted tubule cells have no recognizable cell membrane, a granular acidophilic cytoplasm, and dense pyknotic nuclei. H and E × 200.

solidation and congestion of both lungs with involvement of well over 60 per cent of his pulmonary parenchyma with what appeared to be an extensive bronchopneumonic process. All other organs of this and other animals, both deuterated and control, appeared normal.

Histologic examination: Microscopic examination of the lungs of animal D 1 revealed the presence of an extensive confluent bronchopneumonia. The lungs of the remaining animals were histologically unremarkable. The major findings were limited to the kidneys, salivary glands, and testes of the deuterated animals and are described below.

Kidney: A striking lesion was diffusely but regularly found in the renal cortex consisting of focal tubular damage characterized by loss of tubular structure, clumping and granularity of the cell cytoplasm, and nuclear pyknosis (Fig. 1). The lesions appear to be located in the proximal convoluted tubule; however, work is in progress using more refined techniques which we hope will allow for more precise descriptions of the location and extent of this singular lesion.

Testes: The normal testicular tubular architecture, consisting of an orderly maturation of germ cells, was absent. There was a marked maturation arrest evidenced by the decreased numbers of mature spermia visible in the tubular lumina. A decrease in total cellularity as well as a decrease in number of actively dividing cells was evident. The most striking abnormality was the presence of bizarre giant cells with 4–10 nuclei and granular, hyalinized cytoplasm (Fig. 2). There were numerous degenerate forms with vacuolated, hyalinized cytoplasm, and clumped nuclear chromatin. In many areas, cellular detail could not be distin-

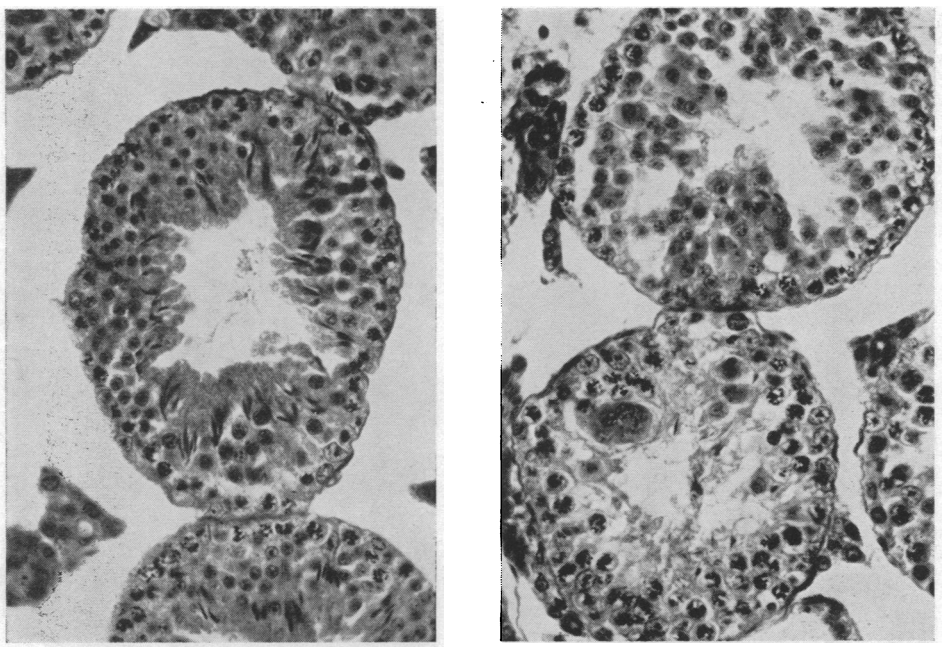

FIG. 2.—(*Left*) Normal testicular tubule of mouse. H and E ×200. (*Right*) Testicular tubule of mouse exposed to heavy water. Mature spermatozoa are scarce, indicating maturation arrest, and one multinucleated cell is present in this tubule. H and E ×200.

guished and only amorphous, eosinophilic clumps of cellular detritus interspersed with occasional nuclear remnants remained.

Salivary gland: Striking alterations were present in the larger excretory ducts of the salivary glands. The ducts appeared to have separated and pulled away from the surrounding acinar tissue. The resulting space between the basal surfaces of the duct cells and the acinar tissue contained amorphous, granular, eosinophilic debris which was similar to the substance present in the lumina of the ducts. Necrosis of duct cells characterized by nuclear pyknosis with clumping and hyalinization of cytoplasm was uniformly present (Figs. 3 and 4).

Discussion.—The clinical observations made by us amply confirm previous studies on the abnormalities encountered during the course of deuteration[5–8] suggesting that the anatomic alterations that we have found have occurred in animals that correspond clinically to those described in the literature as examples of deuterium intoxication. With the exception of the testicular lesion which has recently been described,[9] we know of no previous description of the morphologic changes herein reported.

The first pathological study on heavy water involved the oral and subcutaneous administration of heavy water for 11–21 days to rats in whom the final body water concentration of D_2O achieved was 22.9–41.3 per cent;[10] the authors reported the finding of "parenchymatous degeneration" of the liver and "necrotic changes" in the spleen with all other organs, including kidneys, salivary glands, and testes appearing normal.

The effect of deuteration on water metabolism has been recognized since 1938 when Barbour and Rice[11] reported the occurrence of water retention and transient

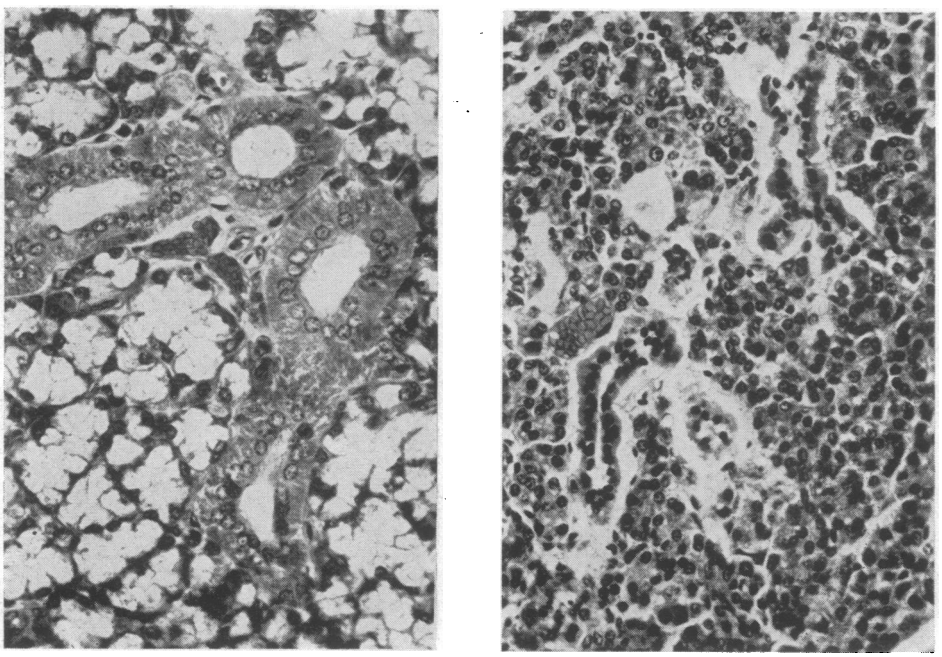

FIG. 3.—(*Left*) Normal mouse salivary gland. H and E ×200. (*Right*) Salivary gland of mouse exposed to heavy water. The ducts are contracted and pulled away from the surrounding glandular tissue. The cells are degenerating and have dense pyknotic nuclei. H and E ×200.

oliguria when mice were given 99 per cent D_2O. Although these workers felt that the effect was secondary to the sympathomimetic action of deuterium in that it could be blocked by ergotoxine, more recent evidence tends to support the hypothesis of a separate renal action of deuteration. In 1957, "tubular destruction" in kidneys which were "large and pale" grossly was described in rats in whom toxicity and death had been produced with heavy water. No further description of this lesion is available, and we have no way of knowing if it resembles the lesion that we have described. In a later paper by the same group,[12] a fall in glomerular filtration rate and renal plasma flow in rats in whom body water deuterium was approaching 30 per cent was described; this alteration was reversible when the animals were given deuterium-free water. In association with many other biochemical abnormalities, a marked rise in serum nonprotein nitrogen has been described.[6] It is possible that the focal tubular necrosis is responsible for renal failure in these animals.

Our finding of destructive changes in the ductular epithelium of yet another organ which is concerned with water transfer, the salivary gland, is rendered more intriguing by the report that in normal pregnant women the "salivary glands concentrate deuterium oxide above the level of serum."[13] As the authors point out, this is the first report of the ability of any mammalian organ to concentrate deuterium oxide.

The influence of heavy water on the generative organs and their products has been known since 1935 when Ussing[14] described the interference with the development of amphibian egg development in various concentrations of deuterium oxide. This effect was confirmed by Lucké and Harvey on Arbacia eggs in 1936.[15] In 1958,

Hughes and Calvin[16] reported the production of 100 per cent sterility in male mice given 30 per cent D_2O in their drinking water for 8 weeks prior to mating. This effect was reversible when the animals were "washed out" with normal water, and was felt to be centered around the late prophase of meiosis on the basis of the time course of the sterility response to deuterium oxide. In a recent paper,[9] Amarose and Czajka reported their findings in the testes of mice maintained on 30 per cent deuterium oxide. They reported the formation of multinucleated cells containing only spermiogenic elements and a complete absence of spermatozoa in the epididymides after 40 days of treatment. In view of the absence of any evidence of necrobiosis, it was the opinion of the authors that meiotic division was complete but that a "block in differentiation of the daughter cells" accounted for the changes that were noted. A "washout" experiment demonstrated the complete recovery of the germ cell architecture. Similar findings were obtained when the testis of a dog maintained on 50 per cent and 75 per cent deuterium oxide was histologically studied. As judged from their descriptions and photographs, the lesions observed by Amarose and Czajka are quite similar to those which we have described; the differences that exist are those of degree rather than quality and may be ascribed to the differing heavy water concentrations and periods of exposure.

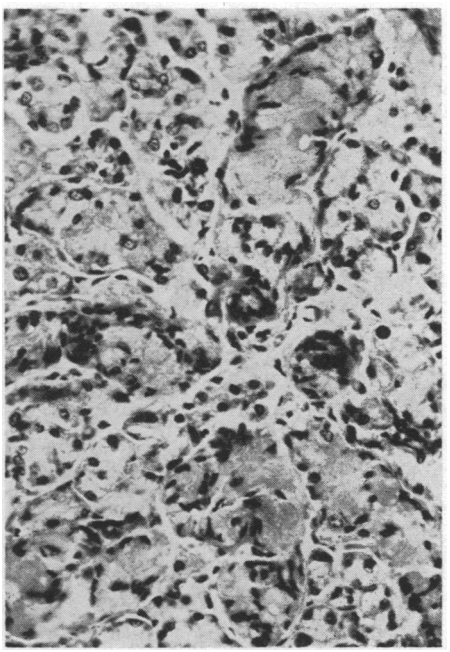

FIG. 4.—Salivary gland of mouse exposed to heavy water. The cells of the demilunes as well as the mucous cells are degenerating. H and E ×200.

The most intriguing question that remains to be answered is by what mechanism isotopic substitution produces the profound alteration in normal structure and function that has been outlined in this and other papers. In so far as our finding of renal tubular damage is concerned, it would be tempting to hypothesize that the localization of cellular damage is simply dependent upon the exposure of the cells concerned to an increased amount of "toxic" material per unit time by virtue of tubular reabsorption of glomerular filtrate. The fact that water is reabsorbed in greatest amount by the proximal convoluted tubule lends support to this idea.[17] In any case, we cannot at this point exclude the possibility of some unique susceptibility of the cells of the renal tubule and excretory ducts of the salivary glands to deuterium oxide—a susceptibility that may be related in some as yet unknown manner to the intimate role of these organs in the water metabolism of the mammalian body.

It should be noted in passing that although we have referred to the toxicity of deuterium the term should be interpreted in only the broadest sense. Borek and Rittenberg[18] have described anomalous growth effects and morphological changes in *E. coli* transferred from one isotopic medium to the other but not in bacteria grown

in pure water on the one hand, or 100 per cent D_2O on the other. The suggestion is therefore made that the effects are not caused "by the rare isotope *per se* but rather by the unusual mixture of isotopes within the organism when they are transferred from the environment of one isotope to another." This argument is clearly applicable in our case and in all experiments with higher organisms where 100 per cent deuteration is technically not feasible.

A definitive explanation of the antimitotic effect of deuteration as exemplified by the testicular lesions described in this paper has not yet been offered. Gross and Spindel[19] have reviewed a number of alternative hypotheses and suggest that the most attractive possibility is a "rigidification" of the mitotic apparatus secondary to small increases in the strength of hydrogen bonds which, taken in their entirety, produce changes in the over-all "viscosity" of the system. Calvin and his associates[20] have emphasized the effect upon macromolecular structure of alterations in hydrogen bonding and have demonstrated significant changes in the strength of hydrogen bonding secondary to replacement of hydrogen with deuterium.[21] In view of the sensitivity of enzyme and, presumably, of genetic function to minute changes in three-dimensional structure the possibility of attributing many of the effects of isotopic substitution to structural changes on the molecular level is an attractive one. The possibility of a direct effect on DNA synthesis also has been investigated. Although inhibition of mitosis by D_2O has been shown to be demonstrable after DNA replication is complete,[19] a primary effect on DNA replication in addition to other effects is still possible. Gross and Harding[22] have shown with autoradiographic experiments that Arbacia eggs immersed in 90+ per cent D_2O do not incorporate tritiated thymidine, thus lending support to the theory of a primary interference by deuterium with DNA synthesis.

Summary.—(1) Young male mice were given varying concentrations of heavy water *ad libitum;* their clinical behavior was observed and at death gross as well as microscopic examination was performed. (2) The clinical course was in accord with descriptions previously recorded in the literature and consisted of hyperactivity superseded by lethargy, coma, and convulsions. (3) Microscopic findings included focal renal tubular necrosis, profound alterations in testicular architecture and cellular morphology, and abnormalities of the excretory ducts of the salivary glands. (4) The relationship of the anatomic changes described to the pathophysiology of deuterium intoxication is discussed.

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[1] Urey, H. C., *Science*, **78**, 566 (1933).

[2] Lewis, G. N., *J. Am. Chem. Soc.*, **55**, 3503 (1933).

[3] Thompson, J. F., *Ann. N. Y. Acad. Sci.*, **84**, 573–781 (1960).

[4] Nief, G., and R. Botter, "Mass spectrometric analysis of simple hydrogen compounds," in *Advances in Mass Spectrometry*, ed. J. D. Waldron (London: Pergamon Press, 1959), p. 515.

[5] Barbour, H. G., and J. Trace, *J. Pharmacol.*, **58**, 460 (1936).

[6] Katz, J. J., H. L. Crespi, T. T. Hasterlik, and A. J. Finkel, *J. Natl. Cancer Inst.*, **18**, 641 (1957).

[7] Katz, J. J., H. L. Crespi, and D. M. Czajka, *Am. J. Physiol.*, **203**, 907 (1962).

[8] Thompson, J. F., *Ann. N. Y. Acad. Sci.*, **84**, 736 (1960).

[9] Amarose, A. P., and D. M. Czajka, *Exptl. Cell Res.*, **26**, 43 (1962).
[10] Dybung, O., G. Guldberg, and K. Hansen, *Klin. Wochschr.*, **17**, 1585 (1938).
[11] Barbour, H. G., and L. E. Rice, *J. Pharmacol.*, **62**, 363 (1938).
[12] Thompson, J. F., and F. J. Klipfel, *Proc. Soc. Exptl. Biol. Med.*, **97**, 758 (1958).
[13] Taggart, H., and F. E. Hytten, *Nature*, **184**, 457 (1959).
[14] Ussing, H., *Skand. Arch. Physiol.*, **72**, 192 (1935).
[15] Lucké, B., and E. N. Harvey, *J. Cellular Comp. Physiol.*, **5**, 473 (1935).
[16] Hughes, A. M., and M. Calvin, *Science*, **127**, 1445 (1958).
[17] Smith, H. W., *Principles of Renal Physiology* (New York: Oxford University Press, 1960).
[18] Borek, E., and D. Rittenberg, these PROCEEDINGS, **46**, 777 (1960).
[19] Gross, P. R., and W. Spindel, *Ann. N. Y. Acad. Sci.*, **90**, 500 (1960).
[20] Moses, V., O. Holm-Hansen, and M. Calvin, *Biochim. et Biophys. Acta*, **28**, 62 (1958).
[21] Calvin, M., J. Hermans, and H. A. Scheraga, *J. Am. Chem. Soc.*, **81**, 5048 (1959).
[22] Gross, P. R., and C. V. Harding, *Science*, **133**, 1131 (1961).

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*DIFFRACTION OF SCALAR ELASTIC WAVES BY A CLAMPED FINITE STRIP**

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Most long wavelength or Rayleigh scattering processes can be described in terms of volume effects: the scattered fields for single scattering are proportional to the volume of the scatterer and to the anomalous excess or deficiency in the physical properties causing the scattering. Rayleigh scattering has, by now, been studied with sufficient detail for wave propagation in a wide number of modes (elastic, acoustic, electromagnetic, etc.) so that it is well understood.

Volume degeneracy is of importance especially in the case of elastic wave propagation in solid matter. In this case we imagine the volume as having collapsed to a surface of finite extent. The surface is a geometrical discontinuity; across this surface, certain stresses or displacements must exist independent of the excitation. Examples for elastic wave propagation correspond to the problems of flaw detection, propagation around glacial crevasses, seismic wave propagation in the upper part of the earth's crust, etc.

In the calculation below, we consider the following example: let an infinite clamped strip be located in an infinite homogeneous medium. The strip is located at $y = 0$, $-1<x<1$, and extends to infinity in both z-directions. Let the system be excited by a plane harmonic wave whose rays lie in planes parallel to the x-y plane, obliquely incident upon the strip. The elastic displacement vector of the incident wave points in the z-direction. This problem is a scalar problem. The fields satisfy the scalar wave equations. The boundary condition at the strip, due to the "clamped" condition, is such that the total field must vanish. This problem also corresponds to the diffraction of acoustic waves by an infinitely massive rigid strip imbedded in a compressible fluid.

A similar problem has been treated for the case of the diffraction of electromagnetic waves of long wavelength by a conducting strip by Groschwitz and Hönl,[1]