Distribution of 4-Iodoantipyrine After Intravenous Injection in the Rat

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The determination of total body water by measurements of the distribution volume of antipyrine was originally proposed by Soberman, Brodie, et al. The applicability of the method has been extended by the use of the more easily determined acetylaminoantipyrine. It has also been suggested that the iodinated derivative of antipyrine labeled with $^{131}$I might be a satisfactory substitute for the parent compound. In one study, it has been shown that the apparent distribution volumes of antipyrine, acetylaminoantipyrine, and 4-iodoantipyrine are nearly identical in human beings as well as in dogs; and another investigation utilizing sheep gave similar results in the comparison of antipyrine and 4-iodoantipyrine. The ease of determination of the radioactive compound has suggested that this material may be an effective substitute for antipyrine in the determination of total body water.

Unfortunately, it has never been shown for iodoantipyrine that it is a true tracer for body water, i.e., that its distribution parallels the distribution of body water. Unless this is shown, as it has been for antipyrine, the validity of measurement of body water with iodoantipyrine must remain in question.

An indication that the distribution of iodoantipyrine did not parallel the distribution of body water was offered by the work of Sullivan and Rose, who found the concentration of radiiodine in brain water to be much lower than its concentration in muscle water after the administration of iodoantipyrine. The authors attributed their results to the possible existence of a blood-brain barrier against either iodoantipyrine or an iodine-containing metabolite of this molecule.

In this laboratory, we have observed substantially the same difference a few hours after iodoantipyrine administration. However, in the early time period after the intravenous injection of the label, the brain takes up considerable quantities of the injected radioactivity, indicating that the barrier, if it exists at all, cannot be to iodoantipyrine as such.

This report is concerned with the distribution of radiiodine among the organs of rats at various times after the administration of iodoantipyrine. The results indicated that the anomalous behavior of the brain is not unique. The use of the $^{131}$I iodoantipyrine for the measurement of total body water is therefore inappropriate. The cause of this behavior has also been studied, and qualitative investigations show it to be due largely to loss of $^{131}$I label from the antipyrine molecule.

**Methods**

DETERMINATION OF APPARENT VOLUME DISTRIBUTION OF 4-IODOANTIPYRINE

Ten to 20 µg of $^{131}$I-labeled 4-iodoantipyrine (Abbott) was injected intravenously into each of four rats anesthetized with pentobarbital sodium. Twenty-µl samples of whole blood were taken at 2, 5, 10, 20, 40, and 80 minutes after injection. The disappearance curve was extrapolated to zero time and the distribution volume calculated as the ratio between the injected dose and the concentration in the blood at zero time. No attempt was

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*Chromatographically assayed to be more than 99 per cent 4-iodoantipyrine.*
made to correct the blood concentration values for blood water.

**DISTRIBUTION OF I$^{131}$ IN ORGANS
AFTER INJECTION OF 4-IDOANTIPYRINE**

Adult female rats of the Sprague-Dawley strain were fasted for 18 hours. At the time of use, they weighed 200 to 400 Gm. One to two $\mu$g. of 4-iodoantipyrine was injected into the femoral vein under pentobarbital anesthesia. Groups of three to four animals were sacrificed at 1, 2, 3, 5, 10, 20, 40, 120, 240, and 1,080 minutes after the injection. The following organs were taken for counting: brain, heart, liver, gut, kidney, skin, and carcass.

The counting of the blood and small organs was accomplished in a Nuclear Chicago DS 5-5 well crystal which displayed its counts through a Nuclear Chicago 132 Computer Analyzer. Larger organs were counted in a large plastic scintillating well (8 inch I.D.) which showed its counts through a Nuclear Chicago Ultrascaler. All counts were compared to those of a standard prepared from the injected solutions and counted in the same systems as the tissues analyzed.

**EVALUATION OF LABEL LOSS FROM 4-IDOANTIPYRINE**

Assuming that the thyroid gland traps only iodide from the circulation and that the stomach also forms a large iodide space, any undue accumulation of label in these two organs following the administration of iodoantipyrine would strongly indicate that a considerable amount of label is lost from the organic molecule. The same statement can be made if the excreted label is found to be iodide ion; that is, any label in the urine (the major excretory route) precipitable by silver ion would be assumed to be split from the original iodoantipyrine molecule.

Two groups of rats received 7.5 and 15 $\mu$g. of iodoantipyrine, respectively, by intraperitoneal injection. In each case, the rats were placed in individual metabolism cages in which urine and feces were separated over the next 24-hour period. Water was made available, but food was withheld from the animals during this period.

At the end of 24 hours, the stomachs and thyroids were counted in the plastic scintillating well referred to above. Urine collections were made on a group of three animals. The urine from the first 8 hours was collected separately from that of the remainder of the 24 hours. Each urine collection was diluted with water to a volume of 25.0 ml. To this was added 10.0 ml. of 0.1 M sodium iodide to provide a carrier for radioactive iodide. The iodide was precipitated with 12.0 ml. of 0.1 M silver nitrate, the precipitate then being washed with approximately 20 ml. of 2.5 N ammonium hydroxide to dissolve any silver compounds other than silver iodide. The mixture was centrifuged, the supernatant decanted, and both it and the centrifuge tubes containing the precipitate were counted in the same manner as were the organs. In all cases, the counts were compared with a standard made up from the same solution as was injected.

**Expression of Results**

The uptake by any organ of I$^{131}$ was compared to the total amount of I$^{131}$ remaining in the animal by the use of the ratio I$^{131}$ in organ / I$^{131}$ in animal. This ratio was in turn compared to the ratio weight of organ / weight of animal. The final ratio, fraction of isotope in organ / fraction of body weight in organ, was used to express the "affinity" of the organ for I$^{131}$. When the ratio has a value of 1.0, the affinity of the organ for isotope is identical with that of the whole body. Smaller values show preferential rejection; larger values show preferential uptake of isotope by the organ.

**Results**

**APPARENT DISTRIBUTION VOLUME OF IODOANTIPYRINE IN THE RAT**

The results in each of four rats were similar to those shown in figure 1. At 1 minute, the concentration in blood was approximately 50 per cent greater than that which was found at equilibrium. Equilibrium was approached rapidly and was essentially complete in 10 minutes. The "equilibrium" concentration was then maintained throughout the period of observation. The appearance of the curve suggested that 10 minutes was required for a complete mixing and that the label was then homogeneously distributed in its final volume. The apparent distribution volume was calculated from the latter portion of the curve extrapolated to zero time. No attempt was made to determine the concentration of the label in terms of the water content of the blood samples. The calculated distribution volume, without correction, was approximately 65 per cent of the body. Making the assumption that the water content of blood was 86 per cent, the calculated distribution volume was 56 per cent of the body.
Typical disappearance curve of iodoantipyrine from the circulation of a rat after a single intravenous injection. The use of this curve in the determination of apparent total body water is also illustrated.

**DISTRIBUTION OF I\(^{131}\) IN ORGANS**

Although the blood disappearance curve had reached a concentration equilibrium at 10 minutes, the inference that complete equilibrium had occurred by this time proved to be spectacularly incorrect. Not only were there major differences in the ratio,

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\text{fraction of isotope in organ} \over \text{fraction of body weight in organ}
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from organ to organ, but also the various ratios had not even begun to approach their final value. Thus at 10 minutes, the liver, kidney, heart, gut, and skin contained far more than the expected amounts of label while the brain and remainder of the carcass contained considerably less. At 1,080 minutes, the only organs containing excessive quantities of the label were the gut and the skin; all other organs showed between 0.16 (brain) and 0.95 (kidney) of the expected amount of label, assuming homogenous distribution through the body mass. These results at various times are illustrated in figure 2.

**LOSS OF LABEL FROM IODOANTIPYRINE MOLECULE**

In a series of seven animals, the percentage of injected counts found in the thyroid after
DISTRIBUTION OF IODOANTIPYRINE

24 hours averaged 10.4, with a range from 5.1 to 12.0; that in the stomach averaged 3.4, with a range from 2.6 to 6.1. After 24 hours, the amount of label in the urine precipitable by silver ranged from 51 to 67 per cent of the injected dose, with an average of 56.3 per cent. From these figures, it is seen that within 24 hours some 70 per cent of an injected dose of iodoantipyrine loses its label which is then distributed and excreted as iodide. It was also observed that in the first eight hours following administration the ratio of \( \frac{\text{organic label excreted in the urine}}{\text{organic label precipitable by silver}} \) equals 2.1 and in the following 16 hours equals 4.7.

Discussion

The fact that at “equilibrium” the iodine moiety of iodoantipyrine is preferentially accumulated in certain organs (gut, skin) and rejected by others is \textit{prima facie} evidence that this material is unsuitable for the measurement of the body water. (Although no water determinations were made on the organs in these experiments, it is reasonable to assume that the water content of each tissue corresponded roughly to its mass; disparities of the order encountered are not within the range of variability to be expected of tissue water content.)

It may be argued that the terminal distribution of the radioiodine reflects the fate of an iodine-containing metabolite of iodoantipyrine, and that prior to the formation of this metabolite in significant amounts the distribution volume of iodoantipyrine does, in fact, correspond to the body water.

The data fail to support this interpretation. During the first minute, as expected, the distribution of the isotope tends to parallel the distribution of the cardiac output. With progressive recirculation, one would expect to find the label redistributing from a “flow-dominated” to a “space-dominated” pattern. If the space explored by the iodoantipyrine molecule corresponded at any time to organ water, it would have been expected that the ratio, \( \frac{\text{fraction isotope in organ}}{\text{fraction body weight in organ}} \), would approach unity, or some value close to unity at some intermediate time. This never happened.

It must, consequently, be concluded that iodoantipyrine is not a suitable indicator for the measurement of body water. The fact that the apparent volume of distribution of the indicator is close to the body water must be attributed to the unhappy chance that there is approximately as much gross overaccumulation of the label in certain organs as there is gross underaccumulation in others.

There are several explanations which might account for this unusual behavior of iodoantipyrine. Two of the more obvious possibilities are: (1) Iodoantipyrine may be selectively dissolved in some other tissue component than water, which is unequally distributed throughout the tissue. (2) The parent mole-
cule may be broken down in part and the distribution pattern of the radioiodine may be a complex function of the distributions of iodoantipyrine, an iodine-containing metabolite of it, and perhaps also inorganic iodide which has been lost from the parent molecule.

Strong evidence in favor of the latter proposition has been offered recently by Sullivan and Rose. These authors noted that the radioiodine in the plasma of animals which had been injected some time earlier with iodoantipyrine was more diffusible than the iodine of iodoantipyrine mixed with plasma in vitro. Presumably, this resulted from the breakdown of the iodoantipyrine molecule into a smaller one or from its conversion into one less firmly bound to the plasma proteins. The authors postulate that I\(^{131}\) may be lost from iodoantipyrine prior to the conjugation of that compound at the 4' position. Reference is also made by Sullivan and Rose to the unpublished observations of another investigator (Hansen) which demonstrated similar label loss in cats. Another study, dealing with the effect of iodoantipyrine on thyroid function, showed that thyroid function is depressed by administration of this compound. The authors believe that this depressant action, rather than being the result of any intrinsic effect of iodoantipyrine itself, could conceivably be due to the conversion of label to iodide which then acts as a depressant.

The qualitative investigations of label loss cited in this report support the previously mentioned studies and clearly indicate that the I\(^{131}\) label is indeed converted to iodide. This would seem to be, therefore, the major factor accounting for the abnormal distribution pattern of iodoantipyrine; for shortly after administration of this compound, iodide distribution masks the true distribution of iodoantipyrine. Although the virtual distribution is similar to that of body water, it is now evident that the label does not measure body water, and any similarity between the expected and found results must be considered a chance similarity depending upon overconcentration in some organs and under-concentration in others due largely to the multiple forms in which the label exists before it is excreted.

**Summary**

The distribution of 4-iodoantipyrine does not parallel that of body water, as evidenced by its overaccumulation in some organs and underaccumulation in others. These disparities occur largely because I\(^{131}\) is lost from the iodoantipyrine molecule, is converted to iodide, and is distributed and excreted as such. Any similarity between the distribution volume of iodoantipyrine and total body water is, therefore, happenstance.

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**References**


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