IODINE RETENTION AND DISTRIBUTION IN RABBITS.

BY WILLIAM A. PEABODY.

This study was undertaken to compare quantitatively the retention and particularly, the corresponding distribution of tissue iodine after *continued* inorganic and organic iodide feeding.

The biological advantage of numerous organic iodine preparations over inorganic iodides often has been demonstrated during the past 25 years. Loeb (1), finding that organic iodide but not potassium iodide tended to accumulate in fatty and lipoidal tissues of rabbits, developed his "Lipotropie" theory. Boruttau (2), however, found iodine in brain after potassium iodide administration; likewise McLean (3), though the increase was much greater after iodized soap. Nardelli (4) found pyroiodone to have greater affinity than inorganic iodide for brain tissue. Abderhalden and Kautzsch (5), feeding dogs; Herzfeld and Haupt (6) with normal men; and Pfeiffer (7), and Klein *et al.* (8), studying thyroid utilization of iodine in dogs, observed better retention with organic iodine. Filippi (9) and Bröking (10) using injections; McLean (3); Zuckmayer (11); and Scharrer and Schwaibold (12), who fed cows and determined the iodine content of the milk, noticed in addition that some (or all) of the iodized proteins studied behaved more like inorganic iodides. The data of Hesse (13), who used increased urinary nitrogen in dogs as a measure of effectiveness after fairly large oral dosages of NaI and organic iodine, show slightly greater effects from some of the latter, with lipoiodin giving a lower but more prolonged increase. The compounds monoiodoisovalerylurea and iododioxyhydropropane proved highly toxic. Jacoby (14) concluded that the Ca salts of certain iodized fatty acids are markedly lipotropic, but that the acids themselves are not.

Iodine Distribution.—The I content of normal rabbits according to Maurer and Ducrue (15) ranges from 3γ % (γ % = 0.001 mg./100 Gm.) for fat, brain 8, lung 9, liver 14, kidney and heart 20, blood 30, bile 40, up to spleen 70, uterus 100, ovary 700 and thyroid $15{,}800\gamma$ %. Baldauf and Pincussen (16) give only 10.9γ % for normal human blood. Reported high values for ovary of the cow were not confirmed by Leitch (Cruickshank), and Cruickshank (17) also found low amounts in fowl ovary. Buchholz (18) detected iodine in all human organs examined, with "fairly large" amounts in adrenals, ovaries, thymus and sometimes the spleen. Sturm and Buchholz (19) reported iodine higher in endocrine organs, human testicles and pancreas, with $^1/_2$–$^2/_3$ of the total in the muscle, $^1/_5$–$^1/_{10}$ in the thyroids. After potassium iodide injection in rabbits McLean (20) found fresh liver to contain as much as 22.8 mg. per cent iodine, part of which appeared in the lipoid fraction; feeding potassium iodide or iodized protein (3) produced relatively greater increase in blood and kidney I than did sajodin. Lesser (21) after feeding rabbits iodized fat and analyzing extracted fat, found greatest concentration of iodine and iodized fat in lung extract. Maurer and Ducrue (15) fed small amounts of inorganic iodine to rabbits, with accumulation especially in skin, lungs and kidneys, most of which excess had disappeared after 96 hours; in human blood, the level returned to normal after 24 hours, but in rabbits and sheep lasted 6–11 days. H. Labbe *et al.* (22) stated that injected iodine showed great affinity for lymphoid tissue, especially spleen and ganglia, with liver next. Valenti (23) noted iodine accumulation in liver and nervous tissue after iodized chaulmoogra oil fed to rabbits. Ishikawa (24) observed as much as fifty-fold increase in spinal-fluid iodine after 1 Gm. potassium iodide ingestion by man (normal 1.2γ %). Lustig and Botstiber (25) concluded that the serum proteins also are a storage place for iodine. Von Fellenberg's (26) fed guinea pigs retained iodine longer in skin, hair and especially muscle. Scharrer (27) after feeding alkali iodides to pigs, found little increase in lungs, muscle, heart and body fat, but large differences in spleen, liver and kidneys.

Elimination.—Maruno (28) noted that rabbits excrete injected organic halogen compounds in the bile more actively than inorganic, but excretion was slight after oral administration. Fricker (29), who fed 1 Gm. of lithium iodide to a man with a biliary fistula, found maximum biliary iodine excretion in 3 hours and 86% elimination in 24 hours. However, the likelihood of reabsorption normally must be considered in connection with biliary excretion. Since fecal and urinary elimination are reviewed extensively in McLean's (3) paper, the question need not be discussed in detail here. The partial dependence of path and rate of excretion upon intermediary metabolism and the specific nature of the latter render the consideration of elimination an individual problem for each compound. The different preparations seem to exhibit wide variations with respect to the extent of I split off in digestion, or subsequently, and the forms in which the I is excreted.

From the foregoing work it may be concluded that the diffusible or loosely-bound iodine of inorganic salts and certain iodized organic compounds is less readily retained for utilization than the more firmly united iodine of iodized soaps, fats or other fatty acid esters, which are more slowly and evenly metabolized. As to distribution within the organism of administered iodine, apparently, as might be expected, the results depend upon a number of factors, such as, the form in which the element is administered, mode of introduction, dosage, duration of administration, species or variety of animal used, normal or pathological state of animal and metabolic or individual differences interrelated with the foregoing. Certainly the concentration of iodine in most if not all tissues can be maintained far above ordinary levels by repeated moderate dosages of almost any absorbable iodine compound.

EXPERIMENTAL.

Pairs of rabbits of the same breed and nearly the same weight, selected without regard to sex, were kept (individually) in metabolism cages. Diet consisted of whole oats and water *ad lib.*, supplemented almost daily with moderate amounts of lettuce, cabbage, beet tops or similar greens, except that animals, 1, 3 and 4 received no greens. In the first three experiments (animals, 1, 3, 4, C-1, C-3 and C-4), the iodized fatty ester or potassium iodide solution was mixed directly with the oats each day. In all subsequent experiments, (indirectly) weighed drops of oil on fresh leaves, and measured volumes of potassium iodide standard solutions dried on the greens, were fed daily, with care to avoid loss, before the bulk of the greenstuff was given. Rabbits 1, 3 and 4 were fed in the spring, the corresponding potassium iodide group C-1, C-3 and C-4 in the summer. As circumstances prevented termination of the first 3 feedings within 2 weeks as originally intended, it was decided to continue until rabbit 1 had consumed one 10-Gm. bottle of oil, and rabbits 2 and 3, one bottle jointly. In all other experiments, feeding was conducted daily at different levels of iodine intake (constant for the experiment, disregarding change in weight of the animal) for 9 days. At the end of the iodine feeding, the animals were kept on the basal diet for 1–4 days additional, after which they were killed by a blow on the neck, bled from the carotid artery and dissected. Tissues were dried in the oven at 100–110° C. for 24 hours. Urine and feces (with waste food) were collected for the total feeding and after period; the solids were partly dried if necessary, weighed, ground, mixed and sampled.

For iodine analysis the procedure of Leitch and Henderson (30) was followed essentially. Although tedious and requiring an exacting technique, the method is recommended by its potential delicacy and the fact that it requires no special apparatus with the possible exception of micro-pipettes and all-nickel crucibles (it was found possible, in fact preferable, to eliminate the electric furnace by heating the crucible inside a second discarded crucible of the same size over a Fisher burner). Solid samples, usually 0.5 Gm., were mixed with 5 cc. of 10% NaOH solution for decomposition and ashing. With urine, samples of 0.5–2 cc. were treated with 0.2–1.0 cc. of caustic; it was found better to omit alcohol extraction in urinalysis, substituting an additional evaporation and ignition if necessary. For analysis of iodized oil, best results were obtained by dissolving a weighed drop of the well-mixed oil in 50 cc. of acetone-alcohol, and treating 1-cc. samples as in urinalysis. By keeping 0.2-, 1- and 5-cc. graduated pipettes at hand, the entire range of iodine concentrations encountered in oil, tissue and excreta analysis could be accommodated, using $N/500$ thiosulphate and sample sizes as indicated.

DISCUSSION OF RESULTS.

The essential data are recorded in the table. Excepting the single analyses recorded for samples of limited size, each reported value is an average of at least two analyses which checked as closely as the nature or amount of the available sample permitted. In Experiment 1, excreta were not collected; these values are omitted for Experiment 3 because of probable loss in collection. Figures for urine and feces are reported together, since a perfect separation was not sought experimentally. The original data shows urinary iodine to be greater, often many times so, for all animals except those of Experiment 6, which received the highest I dosage and eliminated somewhat more in feces than in urine.

A small but consistent advantage in I retention is evident for the organic form of administration, omitting Experiment 4, in which the excessive loss of weight by rabbit 4 renders the per kilo dosage too unequal for a fair comparison. (It should be rembered that rabbit 4 received no green food during the experiment; it is likely that avitaminosis developed in animals 1, 3 and 4.) The lowest dosages used in these experiments was about twenty times the minimum effective dose for humans as determined by Thompson *et al.* (31) in basal metabolism studies. After the more marked results in Experiment 5 as compared with 6, it was thought that reducing the dosage might show still greater differences, but such did not prove to be the case; in fact, the absolute retention after either form of iodide seemed to be a function more of the breed of animal than one of dosage, below greatly excessive levels. The excretions in the extra periods of Experiment 7 are relatively low, though the tissue analyses (*e. g.*, Experiment 8) indicate that much extra iodine must have been retained during those periods. Pregnancy in rabbit C-9 did not seem to affect iodine retention in either direction.

The tissue analyses show no consistent differences, when potassium iodide is compared with iodized oil. Increases occur in all, including brain, but any "lipotropic" effect of the organic iodide is not evident under the conditions employed. Mixed subcutaneous and perivisceral fat from the first pair of animals (Experiment 5) sufficiently adipose to justify collection, upon single analysis yielded little I in either case; the duplicate samples were lost during analysis. The relatively high iodine concentrations in liver, lung and kidneys confirm the consensus of previous authors. The few analyses of gall bladder with bile suggest a possible higher I content after organic administration. Values for heart are unusually high after the larger doses of either compound. Apparently the flooding of the organism with excessive amounts of I causes high penetration of the element into most tissues and organs. Of the tissues studied, the skeletal muscle appears to be most resistant to production of extremely high I concentrations, with the exception of depot fat and possibly brain. The accumulation of such large amounts of I did not produce any obvious gross pathology, unless the much higher incidence of liver infection in the salt-fed animals can be attributed to the fed potassium iodide rather than to chance.

SUMMARY.

Repeated moderate to high dosages of potassium iodide or iodized fatty ester fed to rabbits produced:

1. Demonstrably greater retention after the organic form of iodide.
2. Marked increase of iodine in most tissues and organs; but no consistent differences between the two forms of iodide are characteristic of the values obtained for total iodine in the tissues examined.

Grateful acknowledgment is expressed to the Chemical Foundation, Inc., for a grant which made the work of this laboratory possible.

BIBLIOGRAPHY.

(1) Loeb, *Arch. exptl. Path. Pharmakol.*, 56 (1907), 310; through (20).

(2) Boruttau, *Z. f. exp. Path. u. Therap.*, 8 (1910), 418; through (3).

(3) McLean, *Arch. Internal Med.*, 10 (1912), 505.

(4) Nardelli, *Arch. farm. sper.*, 13 (1912), 381; through *Chem. Abstr.*, 6 (1912), 2461.

(5) Abderhalden and Kautzsch, *Z. exp. Path.*, 4 (1907), 716; through *Chem. Abstr.*, 3 (1909), 1181.

(6) Herzfeld and Haupt, *Med. Klinik*, 7 (1911), 1426; through *Chem. Abstr.*, 6 (1912), 117.

(7) Pfeiffer, *Biochem. Z.*, 215 (1929), 197; through *Chem. Abstr.*, 24 (1930), 1151.

(8) Klein, Pfeiffer and Hermann, *Ibid.*, 225 (1930), 344; through *Chem. Abstr.*, 24 (1930), 5828.

(9) Filippi, *Arch. farm. sper.*, 14 (1912), 1; through *Chem. Abstr.*, 6 (1912), 3286.

(10) Bröking, *Z. exp. Path.*, 8 (1910), 125; through *Chem. Abstr.*, 5 (1911), 103.

(11) Zuckmayer, *Therap. d. Gegenw.*, 54 (1914 ?), 400; through *Chem. Abstr.*, 8 (1914), 760.

(12) Scharrer and Schwaibold, *Tierernährung*, 1 (1929), 37; through *Chem. Abstr.*, 24 (1930), 3541.

(13) Hesse, *Arch. exp. Path. Pharmakol.*, 102 (1924), 90.

(14) Jacoby, *Biochem. Z.*, 74 (1916), 123; through *Chem. Abstr.*, 10 (1916), 1765.

(15) Maurer and Ducrue, *Ibid.*, 193 (1928), 356–371; through *Chem. Abstr.*, 23 (1929), 4488.

(16) Baldauf and Pincussen, *Klin. Wochschr.*, 9 (1930), 1505; through *Chem. Abstr.*, 24 (1930), 5817.

(17) Cruickshank, *Biochem. J.*, 23 (1929), 1044.

(18) Buchholz, *Z. ges. exptl. Med.*, 63 (1928), 188; through *Chem. Abstr.*, 24 (1930), 1893.

(19) Sturm and Buchholz, *Deut. Arch. klin. Med.*, 161 (1928), 227; through *Chem. Abstr.*, 24 (1930), 1421.

(20) McLean, *Arch. Internal. Med.*, 15 (1915), 92.

(21) Lesser, *Arch. Dermatol. Syphilis*, 64 (1903), No. 1; through *Arch. Internal Med.*, 10 (1912), 515.

(22) Labbe, Lortat-Jacob and Boulaire, *Compt. rend. soc. biol.*, 61 (1906 ?), 336; through *Chem. Abstr.*, 1 (1907), 333.

(23) Valenti, *Arch. farm. sper.*, 34 (1922), 108, 118; through *Chem. Abstr.*, 17 (1923), 422.

(24) Ishikawa, *Med. News (Jap.)*, No. 1241 (1928), 1331; through *Chem. Abstr.*, 24 (1930), 1420.

(25) Lustig and Botstiber, *Biochem. Z.*, 220 (1930), 192; through *Chem. Abstr.*, 24 (1930), 3556.

(26) Von Fellenberg, *Ibid.*, 174 (1926), 355; through *Chem. Abstr.*, 21 (1927), 456.

(27) Scharrer, *Z. angew. Chem.*, 41 (1928), 980; through *Chem. Abstr.*, 23 (1929), 907.

(28) Maruno, *J. Exp. Digestive Diseases (Jap.)*, 3 (1929), 939; through *Chem. Abstr.*, 24 (1930), 1430.

(29) Fricker, *Biochem. Z.*, 14 (1909), 286; through *Chem. Abstr.*, 3 (1909), 1301.

(30) Leitch and Henderson, *Biochem. J.*, 20 (1926), 1003.

(31) Thompson, Brailey, Thompson and Thorp, *Arch. Internal Med.*, 45 (1930), 261.

RESEARCH LABORATORY, DEPARTMENT OF CHEMISTRY,
MEDICAL COLLEGE OF VIRGINIA,
RICHMOND, VIRGINIA.

THE ANTHELMINTIC PROPERTIES OF PEPO U. S. P. AND CUCURBITA PEPO.*

BY JAMES S. NEELY AND EDWARD D. DAVY.

The unsettled question of the activity of pumpkin seed as a vermifuge led us to investigate more fully the official drug and to examine the flesh of ripe pumpkin.

The method of determining anthelmintic activity was by the use of earthworms as directed by Sollman (28). The apparent effect is first, one of stimulation, then paralysis, the killing time being taken when the worm ceased to move and did not recover when placed in fresh water.

1. THE EXAMINATION OF PEPO.

One pound of dried pumpkin seed was extracted with petroleum ether to remove fixed oil. This was followed by 75% alcohol until one liter of percolate was collected. Distillation of the percolate under reduced pressure gave a syrupy residue which was diluted to 75 cc. with distilled water and filtered with the aid of kieselguhr. This solution was mildly acid to litmus but highly active, killing in eight minutes. Treatment of this active solution with lead subacetate produced a voluminous precipitate. This was filtered and the precipitate suspended in water and treated with hydrogen sulphide, again filtered and the filtrate distilled under re-

* Scientific Section, A. PH. A., Rapid City meeting, 1929.