this stimulus just as does an operated liver. 2. No significant difference was observed between the rates of regeneration of livers in lobectomized parabions and in lobectomized single animals.

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Metabolites of Thyroxine.* (18595)

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The recognition of thyroxine as the thyroid hormone(1,2) makes it important to study its products of metabolism. In the present work, the radioactive substances occurring in the body after administration of radio-thyroxine were detected by radioautography of paper chromatograms of tissues and excreta.

Methods. Radioactive L-thyroxine was prepared by biologically labeling its iodine as described earlier(3). This radio-thyroxine was injected subcutaneously into (a) 6 intact C_3H mice, (b) 3 thyroidectomized C_3H mice, and (c) 3 intact rats. Each animal of these experimental series was injected every 2 hours over a 12-hour period and sacrificed 13 hours after the last injection. The individual doses varied from 0.05 to 0.15 μg of thyroxine and contained from 1 to 5 μc of radioactivity. The purity of similar radio-thyroxine was found to average 92%(3). The nature of the 8% contamination was investigated by chromatographing the injection material according to the method described below. The contaminants were found to consist of traces of iodide and small amounts of an unidentified substance, hereafter referred to as compound No. 1. To remove these contaminants as completely as possible, a sample of radio-thyroxine was chromatographed, the radio-thyroxine spot cut out, eluted, and the elutant injected into one of the 3 thyroidectomized C_3H mice. This procedure successfully removed all of the iodide and all but minute traces of compound No. 1. For comparison purposes, 12 rats and 1 mouse were respectively injected with 100 and 20 μc of *radio-iodide* by the subcutaneous route. They were sacrificed 48 hours later. Liver, kidney, muscle and feces of each of the injected animals were homogenized in volumes of saline in the ratio of 10 cc per gram of tissue. Plasma and urine needed no homogenization and were used as such. All samples were first extracted with twice their volume of water-saturated butanol and then reextracted with an equal volume of the same solvent. These 2 butanol extracts were combined and concentrated to a small volume of *vacuo*. Aliquots were chromatographed on Whatman No. 1 filter paper along 2 dimensions and the resulting chromatograms were subsequently radioautographed. The technical details and the method used for the identification of the spots on the radioautographs have been reported elsewhere(2,4). Preliminary chromatographic runs yielded similar qualitative results for the starting materials (whole plasma, kidney homogenates in saline, etc.) and the corresponding butanol extracts. Since butanol extracts were easier to handle, they were adopted for routine work.

Results. The injection of *radio-thyroxine* gave similar results in intact and thyroidectomized animals. Plasma radioactivity was predominantly in the form of thyroxine (Fig. 1), but there were also small amounts of iodide and of an unidentified substance located to

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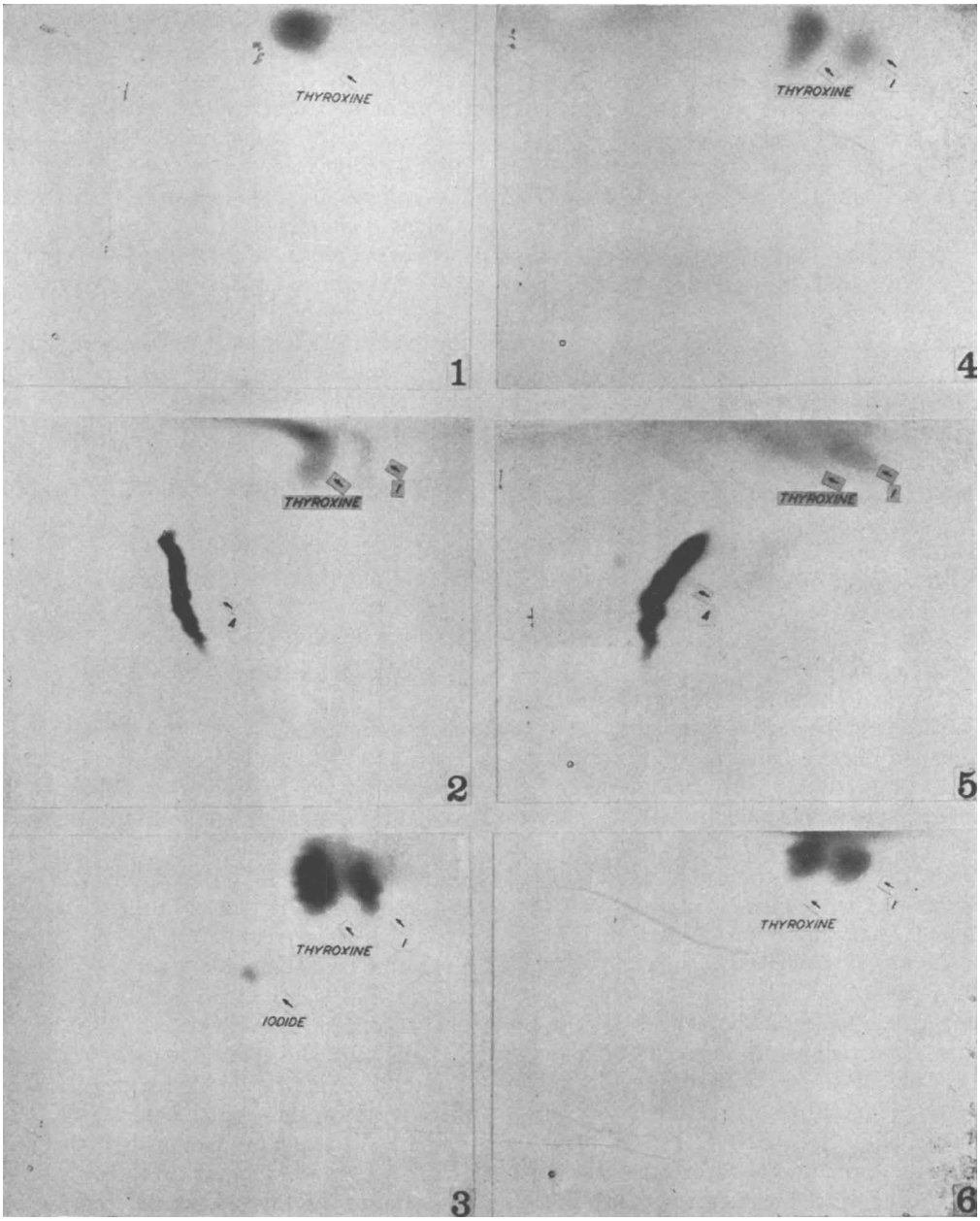

Radioautographs of paper chromatograms. The origins are indicated by the small circles in the lower left hand corner.

FIG. 1. Plasma of C_3H mouse given labeled thyroxine over 24 hr period.
FIG. 2. Kidney of same animal.
FIG. 3. Feces of same animal.
FIG. 4. Plasma of C_3H mouse given radioiodide 48 hr previously.
FIG. 5. Kidney of same animal.
FIG. 6. Feces of same animal.

the right of thyroxine and referred to as compound No. 1. The kidney contained thyroxine, compound No. 1, traces of iodide, and an unknown compound—referred to as No. 4—which was located to the middle left of the radioautographs and was particularly abund-

ant in mice (Fig. 2). The liver showed an intense thyroxine spot and moderate spots for iodide, compounds No. 1 and No. 4. Muscle and feces contained thyroxine, compound No. 1, and small amounts of iodide (Fig. 3), with the possibility of a faint spot corresponding to compound No. 4. The urine yielded chromatograms which differed from the others by the absence of thyroxine and compound No. 1, but iodide—the only substance identified with certainty—was present in large amounts.

Forty-eight hours after injection of *radio-iodide,* the picture was more or less similar to that obtained after radio-thyroxine administration. Thyroxine itself was found in plasma (Fig. 4), organs (Fig. 5), and feces (Fig. 6), but not in urine. Compound No. 1 had the same distribution (Fig. 2, 4 and 6). A large amount of compound No. 4 appeared in the kidney, especially in that of the mouse (Fig. 4). The urine contained very large amounts of iodide.

Discussion. After *radio-thyroxine injection,* 4 labeled substances were found: (1) thyroxine—in plasma (Fig. 1), organs (Fig. 2), and feces (Fig. 3); (2) iodide—mainly in urine(5,6); (3) compound No. 4—predominantly in the kidney (Fig. 2); and (4) compound No. 1—with the same distribution as thyroxine. Compounds No. 1 and No. 4 were different from all so far investigated iodine compounds of biological significance.

The possibility that these substances were artefacts due to the radiochemical effect of the β-rays from radio-iodine may be discounted, since small doses of radioactivity (less than 5 μc of radiothyroxine were used. Similarly, the possibility that the thyroid gland played a role in the formation of these substances may be dismissed, since the results obtained in thyroidectomized animals did not differ from those in intact controls. It was, therefore, concluded that the substances observed were metabolites of thyroxine and were formed in peripheral tissues.

The origin of compound No. 1 had to be especially considered. Since this substance was shown by chromatography to contaminate the radio-thyroxine administered to the animals, the spots for compound No. 1 found in chromatograms of tissues and feces might simply have been due to a retention of the injected contaminant. However, while these spots were much weaker than the thyroxine spots in the injection material, they were often just as intense in tissues. Thus, if they were due to a retention in the body of the compound No. 1 contaminating the injected thyroxine, this retention would have to be far more efficient than that of thyroxine. This did not seem to be the case, since both substances were equally excreted in feces (Fig. 3). Therefore, the alternative possibility, that is, that compound No. 1 was derived from thyroxine, provided a more satisfactory explanation for the large amounts of this compound found in tissues and feces.

Forty-eight hours after *radio-iodide injection* the same four substances—thyroxine, iodide, compounds No. 1 and 4—were found in tissues (Fig. 4 and 5) and excreta (Fig. 6). Presumably, the injected radio-iodide had first been transformed into radio-thyroxine by the thyroid; and this endogenous radio-thyroxine, following release into the circulation(1,3,6), had been metabolized to yield the other iodine-containing substances detected. The previous finding of compound No. 1 in thyroid tissue and plasma(4) raised the possibility that this substance, like thyroxine, was released from thyroglobulin and passed into the circulation to form a second thyroid hormone. However, the alternative possibility, namely, that compound No. 1 was a product of thyroxine metabolism occurring wherever thyroxine was present, even in the thyroid, would seem to be in better agreement with the results obtained with radio-thyroxine.

Summary. Following injection of I^{131} *labeled L-thyroxine* into intact and thyroidectomized mice and intact rats, tissues and excreta were investigated by radioautography of paper chromatograms of their butanol extracts. Four substances, namely thyroxine, iodide and two unidentified iodine containing substances referred to as compounds Nos. 1

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and 4 were found. The same four substances were likewise found in organs and excreta taken from intact mice and rats injected 48 hours previously with *radio-iodide,* or in other words, after a time interval sufficient for endogenous production of labeled thyroxine in the thyroid and its subsequent distribution in the body. These findings suggest that thyroxine is metabolized to yield at least the two compounds Nos. 1 and 4 as well as iodide.

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In vitro Inhibition of Radiophosphate Uptake and Growth of a Neurotropic Virus by 5-Chlorouridine.* (18596)

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It has previously been reported(1-3) that infection of minced 1-day-old mouse brain with Theiler's GD VII strain of mouse encephalomyelitis virus increases the incorporation of $P^{32}O_4$ into the phospholipide and total protein-bound phosphate fractions. The increased turnover in the total protein-bound phosphate fraction was due primarily to the increased turnover of the ribonucleic acid fraction, the slower turnover of desoxyribonucleic acid phosphorus being unaffected by virus propagation(1). These experiments suggested that virus propagation was intimately associated with ribonucleic acid metabolism, and raised the question of what effects a nucleoside antagonist might have on virus propagation and nucleic acid metabolism. Previous studies have shown that 8-azaguanine had little effect on the growth of Theiler's virus in minced 1-day-old brain or on $P^{32}O_4$ metabolism of this system(3). Benzimidazole, however, inhibited virus growth and $P^{32}O_4$ uptake(3). 5-Chlorouridine has been shown to be a potent uridine antagonist in Neurospora(4). Preliminary experiments showed that 5-chlorouridine inhibited the propagation of Theiler's GD VII virus and led to the present experiments. In these experiments we have investigated the effects of 5-chlorouridine and of uridine, alone and in combination, on the uptake of $P^{32}O_4$ by the acid-insoluble fractions of minced 1-day-old mouse brain and on the propagation of Theiler's GD VII virus in this tissue.

Methods. The methods employed have been described in previous papers(1-3). Minced brain tissue, 40 to 60 mg, aseptically removed from 1-day-old mice was added to sterile 50 ml Erlenmeyer flasks containing 2.5 ml of Simms' solution containing 1 microcurie of $P^{32}O_4$. Uridine and 5-chlorouridine§ were added in concentrations of 3 mg per ml and 1 mg per ml of Simms' respectively. The pH was adjusted to 9 with dilute NaOH. The preparations were inoculated with virus or control supernatants, and brought to a final volume of 3 ml with Simms' solution. The flasks were closed with rubber stoppers and incubated 24 hours without shaking at 35°C in an atmosphere of air. At the termination

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