of DMCT was evident when short but not long wave UV light was used.

It is significant that photosensitization of cultured human cells was produced by concentrations of DMCT as low as 1.6 μg per ml. This is a level which is easily produced in the serum of patients to whom this drug is administered(7,8). The data suggest a possible explanation for the clinical observation that photosensitivity is a common untoward effect of administration of DMCT and is not a problem when MC or TC is used (1-5,7).

Summary. Exposure of cultured human amnion cells to some tetracycline compounds produced slight suppression of growth. When drug treated cells were subjected to irradiation with short-wave (2537Å) UV light, those to which demethylchlortetracycline had been added were severely damaged, the effect being moderate at a concentration of 1.6 μg per ml of the drug and marked at a level of 6.4 μg per ml. Methacycline and tetracycline were without significant effect. Exposure to long-wave (3960Å) UV light did not increase the slight damaging effects of the antibiotics on cells. The phenomenon was observed at concentrations of demethylchlortetracycline commonly present in the serum of patients treated with this agent and appears to be due to cellular injury produced by irradiation on cells which have been sensitized by exposure to drug.

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Autoradiographic Investigations of ^{14}C-labelled Thalidomide and Glutethimide in Pregnant Mice. (29294)

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Comprehensive investigations by Gross, Hoffmann, Keberle and Tripod(4) have demonstrated that numerous α-di-substituted glutarimides have a central nervous system depressing effect. Due to favorable pharmacological properties, the α-phenyl-α-ethyl-glutarimide was introduced in therapy as a hypnotic agent with the name of glutethimide.

By substituting nitrogen in the α-position of glutarimide, derivatives of glutamic acid are formed which possess essentially different chemical and pharmacological properties. Among them the compound N-phthalyl-d-l-glutamimide, known as thalidomide, showed central nervous system depressing action both in experimental animals (Kunz, Keller and Mückter(6) and in man. In large doses, however, it did not produce general anesthesia or lethal poisoning. Nor did the substance possess any anticonvulsive effect which is usually found associated with hypnotic agents. When introduced in therapy, thalidomide was therefore regarded as promising until the well-known toxic effects stopped its further use.

Recently the metabolic fate of glutethimide and thalidomide was investigated (Keberle, Hoffmann and Bernhard,(5); Faigle, Keberle, Riess and Schmid(3); Beckmann(1,2)). Glutethimide was found to be metabolized by oxidative processes, probably in the liver microsomes. Thalidomide however is transformed into various carbonic acids by hydrolytic reactions. As an attempt to elucidate

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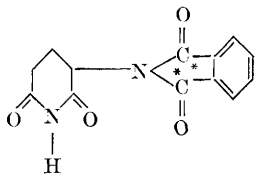
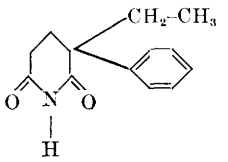
further their fate in the body, the distribution of the two substances labelled with ^{14}C has been compared, using whole body autoradiography.

Methods. ^{14}C-labelled thalidomide and glutethimide were prepared in the laboratories of Ciba, Basle. The specific activity of both substances was 0.7 μC/mg. The position of the radioactive carbon (*) is illustrated by the following formula:



Thalidomide



Glutethimide

For each substance 3 pregnant mice in the late gestation state (2 days before expected parturition) were used as experimental animals. One of the mice was given ^{14}C-thalidomide in water suspension by stomach tube, the second was given the preparation suspended in oil (oleum arachidis) also by stomach tube, while the third mouse was injected subcutaneously with an oil suspension. Glutethimide was administered in the same manner. In each the dosage was 0.5 mg, corresponding to 0.35 μC per gram body weight. The animals given glutethimide went into hypnosis but those to which thalidomide was administered did not. The mice which received the ^{14}C-thalidomide perorally were killed 4 hours after its administration. The mouse which was injected subcutaneously, was killed 8 hours after administration. Corresponding time intervals for the animals obtaining glutethimide were 2 hours and 4 hours, respectively. The mice were sacrificed by immersion in acetone cooled to about —70°C with solid carbon dioxide.

Autoradiography was done according to methods previously described (Ullberg(7)). The frozen animals were transferred to a refrigerated room kept at —10°C and mounted on large stages fitted to a sledge microtome. Sagittal 20 μ sections through the whole animal were taken and dehydrated at —10°C.

Exposure was made by apposition against X-ray Film (Structurix, Gevaert). The exposure time was about 2 weeks.

Results. 1. *^{14}C-thalidomide given orally in water.* The distribution picture is very even except that the gastrointestinal tract contains a rather large amount of radioactivity. A slight accumulation can also be seen in the renal pelvis, the liver and the biliary system, probably due to excretion. The myocardium diverges by showing a slightly higher level, and the compact bone, dentin and enamel by showing a significantly lower level; but in the rest of the body radioactivity is distributed in a remarkably homogeneous manner.

Thus no difference in radioactivity level can be seen between the blood and tissues, and the brain has about the same concentration as the skeletal muscles. The distribution within the brain is very even although the grey matter shows a slightly higher concentration than the white. Peripheral nerve trunks show a slightly, but rather insignificantly, higher uptake than the brain.

In the spleen no difference can be seen in ^{14}C-concentration between red and white pulp; and the ovary, the copora lutea, the walls and liquor of the folllicles all have about the same activity. This also applies to the cortex and medulla of the adrenal. No apparent accumulation can be seen in fat. In the lens of the eye, a peripheral zone shows a level similar to that of other tissues, while no radioactive substance can be observed in the central part of the lens.

The placenta and fetus show about the same ^{14}C-concentration as the maternal tissues. Distribution within the fetuses is also very even. A slight accumulation can be seen in the gastric mucosa and lumen as well as in the renal medulla of the fetus. Fetal bone and cartilage show the same degree of ^{14}C-uptake as the other tissues.

2. ^{14}C-thalidomide given orally in oil. The distribution patterns were very similar to those found when thalidomide was given orally in water. No significant difference could be noted.

3. ^{14}C-thalidomide injected subcutaneously in oil. The autoradiograms showed a slightly

lower concentration of radioactivity in the tissues probably due to the longer interval before sacrifice after administration in this case (8 hours instead of 4).

The distribution picture was very similar to that found after oral administration. The same remarkably uniform distribution was seen. The concentration of radioactivity in the gastrointestinal tract was in relation to total activity almost as high as after oral administration. Most of the activity in the intestinal lumen appeared to be derived from the biliary duct. Signs of excretion through the gastric mucosa were also observed.

4. ^{14}C-glutethimide, given orally in water. The distribution picture is not as uniform as for thalidomide. Specific uptake is seen in liver and fat (also "brown fat"), mammary

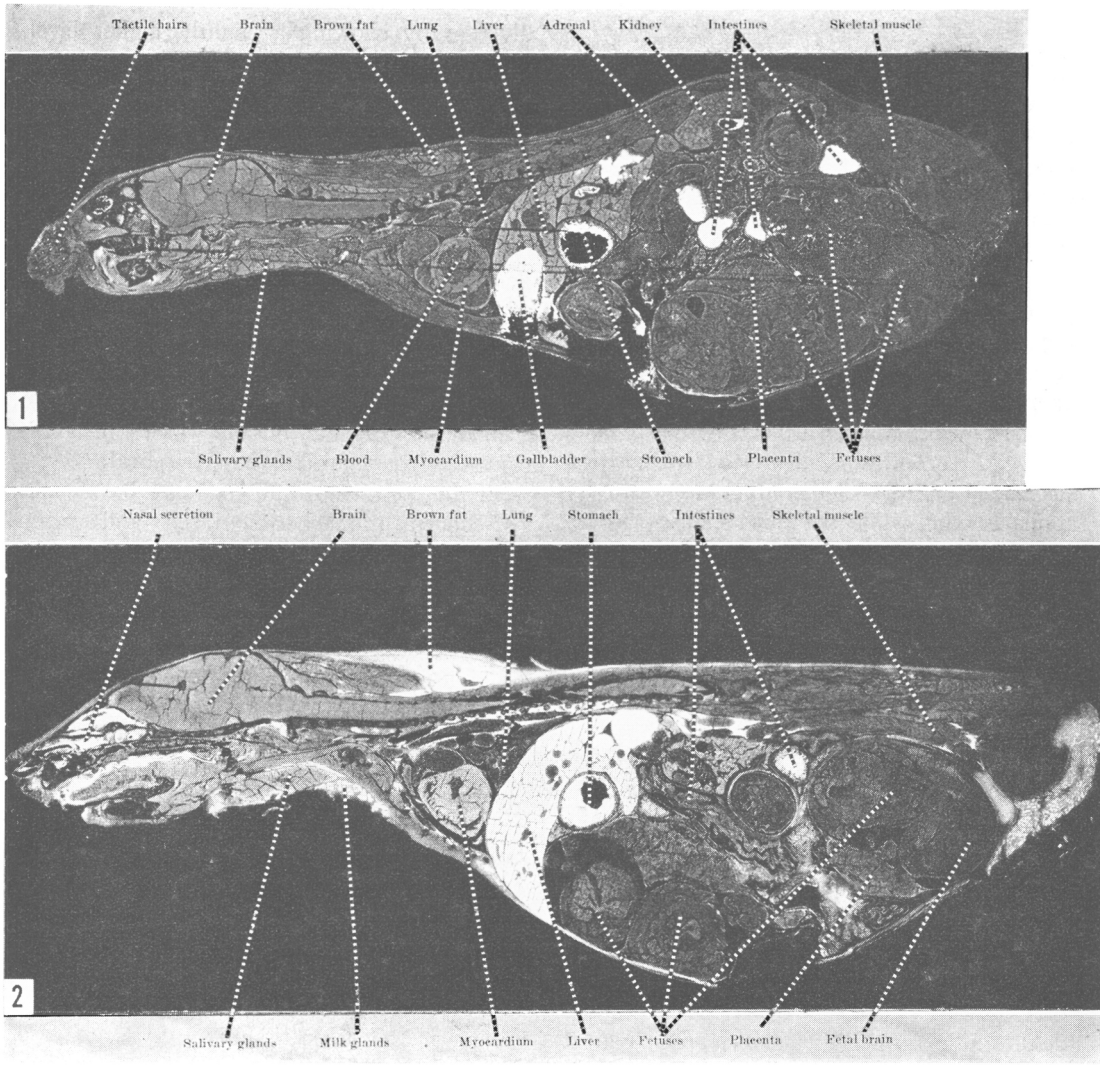


FIG. 1. Autoradiogram showing distribution pattern in a pregnant mouse 4 hr after oral administration of ^{14}C-thalidomide. White areas correspond to high concentration of radioactive substance. Note very even distribution in most maternal and fetal tissues. Slight accumulation is seen in maternal liver; high concentration in gall bladder, gastrointestinal tract and renal pelvis.

FIG. 2. Autoradiogram showing distribution pattern in a pregnant mouse 2 hr after oral administration of ^{14}C-glutethimide. Note accumulation in maternal liver, fat, walls of large blood vessels, mammary glands and myocardium. High concentration is seen in gastrointestinal tract and nasal excretion.

glands and Harders glands. Accumulation can also be seen, probably in connection with excretion, in the nasal conchae, renal medulla and pelvis and biliary ducts. The brain and pituitary gland of the mother show higher concentrations than do the skeletal muscles but lower than fat. Within the brain the distribution is rather even, but slightly higher concentrations are found in grey than in white matter. The difference between grey and white matter is more pronounced for glutethimide than for thalidomide.

The myocardium and walls of the large arteries show higher concentrations than the skeletal muscles. The concentration in blood is lower than in the tissues, except mature bone, which shows hardly recognizable amounts of radioactivity. The level in bone marrow is similar to that of skeletal muscles. As for thalidomide, activity could be seen only in a peripheral zone of the lens of the eye. An accumulation in hair follicles was also noticed.

The fetuses and placentae show a slightly lower concentration of ^{14}C than the maternal tissues and a more even distribution. Slight accumulation is observed in the fetal liver, myocardium, gastric mucosa and renal medulla. The fetal brain, fetal bone and cartilage show about the same concentration as other fetal tissue and an even distribution.

5. ^{14}C-glutethimide given orally in oil. No significant difference was seen when the glutethimide was given in oil instead of water.

6. ^{14}C-glutethimide injected subcutaneously. After subcutaneous administration the concentration in the liver was lower compared with other tissues than after oral application. Just as was the case for thalidomide a relatively high amount of radioactivity was seen in the stomach and intestine after glutethimide administration. The main part of this activity very probably results from excretion into the bile. The distribution pattern is otherwise not noticably different from that seen after oral glutethimide application.

Discussion. These results show that after administration of ^{14}C-thalidomide the radioactivity penetrates the placenta and appears in the fetus in about the same concentration as that observed in the mother. Within the fetus it also is seen in the developing bone. Radioactivity also appears in nerve trunks of the adult.

This points out the possibility that the toxic effects which have been ascribed to the drug may be derived from a local damaging effect in the affected regions. No specific accumulation in these sites has, however, been observed. In fact, examination of practically all tissues and body fluids showed no significant specific accumulation at any site other than those related to excretion.

The distribution pattern after injection of ^{14}C-glutethimide differed in several respects from that found with ^{14}C-thalidomide. Thus, there was a higher degree of uptake from the blood into the tissues and a specific accumulation was found in tissues such as body fat, brain of the adult, myocardium, walls of the large arteries and in the mammary glands. The specific uptake in lipoid rich tissues may be attributed to the higher affinity of glutethimide for lipids. The distribution between water and oleum arachidis was found for thalidomide to be about 1:4 and for glutethimide about 1:40.

The different metabolic fate of the two substances should also be considered in this connection. According to the investigations cited in the introduction the metabolism of the two compounds occurs not only through different biochemical reaction mechanisms, but may also take place in different tissues. Further investigations concerning absorption, distribution and elimination after single doses and repeated administration of both the original substances and their metabolites should be made. Investigations in different states of pregnancy should also be of value.

Summary. The distribution in pregnant mice of ^{14}C-labelled thalidomide and glutethimide was investigated autoradiographically. After administration of ^{14}C-thalidomide the radioactivity was found to penetrate through the placenta and to be distributed very evenly in both mother and fetuses. After administration of ^{14}C-glutethimide a specific accumulation was seen in fat, brain, myocardium, blood vessel walls and mammary glands.

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New Metabolizable Immunologic Adjuvant* for Human Use. I. Development and Animal Immune Response. (29295)

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A principal purpose for employment of an immunologic adjuvant is to achieve a more durable immunity of a higher level employing a smaller antigenic mass in a fewer number of doses than could be achieved by administration of the equivalent aqueous antigen. Development of an immunologically satisfactory and pharmacologically acceptable adjuvant seems a prime essential to preparation of workable multivalent killed virus vaccines such as are required to be effective and practical against the viral respiratory disease complex. Freund's incomplete mineral oil adjuvant without added Mycobacteria(1) has enjoyed widespread use in experimental vaccines. Although highly satisfactory from the standpoint of achieving persistent high level of antibody following immunization(1-6), routine application of Freund's adjuvant to man has not been achieved to date likely because of a large body of data relating to induction of potentiation of plasma cell tumors in BALB/c mice, of induction of autoimmune reactions, and of formation of disseminated focal granulomata in animals injected with mineral oil alone or with complete or incomplete adjuvant with or without added antigens(1,7-16). These effects found in animals are likely without significance in the human being since studies in man have indicated no significant adverse effect referable to the adjuvant other than local reactions at the injection site(17) or some apparent increase in hypersensitivities in inoculated individuals(18,19). Perhaps the principal objection to Freund mineral oil adjuvant acceptance, albeit theoretical, is the apparent long-term persistence of the mineral oil(1). It seemed important, therefore, to seek an effective adjuvant which would be free of saturated aliphatic hydrocarbons.

Work conducted in these laboratories since 1958‡ has been directed to development of a safe and effective immunologic adjuvant for human use which would be comprised of readily metabolizable components which fail to give evidence of significant adverse effects in the human and in animal species. These investigations culminated in development of a stable water-in-oil preparation which is called adjuvant 65 and which comprises aqueous antigens in peanut oil, aluminum monostearate, and mannide monooleate (Arlacel A). Each of the components of the adjuvant enjoys the precedent of long-term use in the human species(1-6,20-24). Additionally, extensive tests carried out to date of the new

* Adjuvant 65.

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‡ Earlier investigations of immunologic adjuvants were conducted in these laboratories by Drs. M. R. Hilleman, J. McKenna, J. D. Mullins, K. Stevens, T. Stim, B. Sweet, A. A. Tytell and A. F. Woodhour.