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Lehigh University, Ph.D., 1974
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SYNTHESIS AND EVALUATION
OF POTENTIAL
PANCREATOTROPHIC RADIOPHARMACEUTICALS

by

H. Donald Burns

A Dissertation
Presented to the Graduate Committee
of Lehigh University
in Candidacy for the Degree of
Doctor of Philosophy
in
Chemistry

Lehigh University

1974

CERTIFICATE OF PRESENTATION

This dissertation is respectfully submitted to the Graduate Faculty of Lehigh University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

H. Donald Burns

A CERTIFICATE OF APPROVAL

Approved and recommended for acceptance as a dissertation in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 22, 1974
Date

Professor in Charge

Special Committee directing work of Mr. H. Donald Burns:

Dr. Ned D. Heindel, Chairman

Dr. Thomas E. Young

Dr. William E. Ohnesorge

Dr. Keith J. Schray

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ABSTRACT

In the search for a γ-emitting radiopharmaceutical suitable for imaging the pancreas by scintillation scanning or gamma-camera methods, a number of new candidate agents were synthesized and evaluated. These agents were structural models of known hypoglycemics, hyperglycemics and pancreatic stimulatory hormones.

Using tolbutamide and/or glycodiazine as the prototype pancreatic hypoglycemic, 5 analogues were prepared which would be capable of carrying ^{99m}Tc in cationic state as a chelating ligand. In a rat glucose tolerance assay, two of these were found to display potency, one equal to tolbutamide itself, and these two were then selected for more detailed tissue distribution studies.

In the course of preparing the labeled chelate, several literature methods for preparation of such ^{99m}Tc-tagged agents were evaluated. It was determined that the most suitable procedure for labeling compounds of the type described herein involved a stannous chloride reduction of pertechnetate ion in the presence of the desired ligand, followed by purification via gel filtration chromatography. Tissue distribution studies on these chelates showed a very high uptake in the kidney and a pancreas/liver ratio of less than 1.0, indicating that such chelates would be unsuitable for imaging normal pancreatic tissue.

Gastrin tetrapeptide -- the carboxyl terminal tetrapeptide from the pancreatic stimulatory hormone gastrin -- was also

labeled by the procedure described above and tissue distribution studies performed. Again, the highest concentration was found to be in the kidney, and a pancreas/liver ratio of less than unity was obtained.

For a model hyperglycemic agent, the diabetogenic antibiotic streptozotocin was selected as a prototype for synthetic modification. Initial studies showed that streptozotocin itself could not be directly labeled by chelation or iodination. Therefore a number of analogues of this antibiotic were synthesized which would be expected to label with ^{131}I (either by iodide displacement of a labile chlorine atom or by electrophilic iodination with iodine monochloride. One of these analogues displayed a unique physiological effect in that it caused a transient diabetic effect in mice, with glucose metabolism returning to normal after several days. This same compound, while giving a pancreas/liver ratio of approximately one in normal rats, yielded a pancreas/liver ratio of approximately three in hamsters with adenocarcinoma of the pancreas during preliminary testing. Furthermore, a tumor to benign tissue ratio of nearly three was also obtained. These results are viewed as quite promising since such localization would result in the tumor appearing as a "hot spot" on a scintiphoto or scan.

INTRODUCTION

A. Pancreatic Cancer

Carcinoma of the pancreas is the fourth leading cause of cancer deaths in this country.[1,2] It is responsible for the death of approximately 7,000 persons each year in the United States.[3] In a recently released epidemiologic study, Wynder and co-workers[1] have reported that this form of cancer is most predominant in males, especially those under the age of 50. The report also indicated a significant association with cigarette smoking and a suggestive association with cigar smoking. In females this disease seems to be related to early onset diabetes. Based on this study and additional pathologic data, Wynder has suggested a working hypothesis as to the cause of pancreatic carcinoma: "The bile might contain carcinogens, originating from tobacco, occupational environment and possibly diet,and causes cancer on reflux into the pancreatic duct."[1]

The prognosis for patients diagnosed as having pancreatic carcinoma is rather bleak. The median survival time for such patients has been reported as 908 days[4] post diagnosis and only 1.5% of these people are alive at the end of 5 years.[5] It is quite evident that very little progress has been made in combating this disease, especially in view of the fact that in 1940 the 5 year survival rate was 1.0%.[5] As Blau has pointed out, "During the past 20 years surgical techniques for the resection of carcinoma of the pancreas

have been perfected; however, few cases are diagnosed sufficiently early to be suitable candidates for operation."[3] This poor survival rate is not due to the non-availability of adequate treatment procedures, but reflects the lack of a reliable diagnostic technique.

In a recent study conducted by G. R. Youngs and co-workers[6] of the Royal Free Hospital in London evaluating the four main tests for pancreatic carcinoma, it was found that the most reliable procedure was imaging of the pancreas with the radiopharmaceutical ^{75}Se-selenomethionine coupled with a ^{99m}Tc-sulfur colloid scan to allow subtraction of interfering background radiation from the liver. Such organ imaging procedures have undergone extensive development during the last ten years and are rapidly becoming the standard front-line attack against cancer. These techniques, in many cases, allow the physician to precisely locate tumors and metastases prior to radiation therapy, surgery or chemotherapy.

B. Organ Imaging with Radiopharmaceuticals

The term organ imaging radiopharmaceuticals has been defined broadly as "those drugs which are specifically used for diagnostic purposes by reason that their penetrating radiation supplies fundamental information of both normal and pathological processes."[7] In general, a radiopharmaceutical consists of a γ-emitting nuclide in ionic form or attached to an organic carrier molecule by chelative or covalent bonds. These agents can be administered by one of three main routes: intravenous, intrathecal or oral. Ideally, the isotope-carrier combination remains intact and is transported

to and concentrated in a desired target organ. This organ is then visualized by the use of external detectors such as the rectilinear scanner or gamma camera. The localization process may be due to one of several mechanisms: active transport,phagocytosis, cell sequestration, capillary blockade, diffusion or compartmental localization. There are examples of radiopharmaceuticals operating by each of the above mechanisms currently in use.

Counsell and Ice[8] have described two basic approaches to the design of new radiopharmaceuticals. The first, and most widely used until recently, is the empirical method. This procedure involves injection of readily available nuclides in various forms and observing the resulting distribution. This method has led to the development of a number of current diagnostic procedures such as the use of ^{99m}Tc-pertechnetate for the detection of lesions of the brain and ^{67}Ga-citrate for the detection of soft tissue tumors. The second and somewhat more rational procedure is the mechanistic approach. This involves choosing an appropriate compound which (based on pharmacological or biochemical data) is believed to localize in the target organ. The carrier molecule is then modified in some manner which allows it to be labeled with an externally detectable nuclide.

The choice of the radionuclide depends on several criteria. Obviously, the nuclide must be one which can be bound, in some form, to the organic carrier moiety and it should not modify the carrier's physiological distribution. Because of the increased use of the

gamma camera, the ideal nuclide should decay by the emission of a gamma photon having an energy between 100 and 200 Kev.[8] If a rectilinear scanner is to be used, adequate organ images can be obtained with gamma energies in excess of 500 Kev.

The half-life should be of sufficient time to allow the preparation, administration, localization and detection of the radiopharmaceutical but short enough to minimize the absorbed radiation dose to the patient. The emitted energy should be as nearly monoenergetic as possible with a minimum of nonpenetrating beta particles. Finally, it should be available in quantity and in a form which makes its use practicable. Some of the most commonly used radionuclides are listed in Table I with their pertinent physical constants.

Table I

Physical Constants of Commonly Used Radionuclides*

Nuclide	Principle Photon (Kev)	% Abundance	Half Life
^{52}Fe	165	100	8.2 hr
^{99m}Tc	140	90	6.03 hr
^{123}I	159	83	13.3 hr
^{125}I	35 (and Fe x-rays)	140	60 d
^{131}I	364	82	8.04 d
^{113m}In	393	64	104 min
^{75}Se	265	60	120 d
^{198}Au	412	95	2.7 d
^{67}Ga	296		78 hr
^{197}Hg	77 (and Au x-ray)	118	2.7 d
^{203}Hg	279	77	46.9 d
^{75}Se	265	60	120 d
^{18}F			

* Taken in part from PDR ref #16

C. Pancreatic Radiopharmaceuticals

Among the functions of the pancreas is the biosynthesis of the hormone insulin and the digestive enzymes (principally trypsin, chymotrypsin and carboxypeptidase A and B). One of the earliest attempts at development of a pancreatotropic radiopharmaceutical sought to capitalize on the synthesis of insulin by the pancreas. Crystalline insulin contains 0.4% zinc. In 1959 Meschen and co-workers evaluated several radiolabeled zinc (^{65}Zn) compounds.[9] Their rationale was that the nuclide would concentrate in the pancreas during insulin synthesis. Although this approach was quite reasonable, pancreatic concentration was too low to be of clinical value.

Based on the fact that toluidine blue (I) causes visual coloration of the pancreas when injected intravenously, a number of investigators have attempted to label this dye for use as a potential pancreas scanning agent. Attempts at labeling this molecule with both iodine-131 and technetium-99m yielded compounds whose organ distributions were unsuitable for pancreas visualization.[10,11]

$(CH_3)_2N$ S NH_2 N CH_3 H

I

CH_2CHCO_2H NH_2 HO ^{131}I

II

Tissue distribution studies with carbon-14 and sulfur-35 labeled amino acids have demonstrated that these compounds concentrate in the pancreas shortly after intravenous injection.[12,13,14] It has been reported that 12.5% of the administered dose of ^{14}C-DL-tryptophan was found in the pancreas one hour after injection.[3] Blau has commented that: "The very rapid utilization and destruction of the digestive enzymes in comparison with other body proteins requires a rapid rate of synthesis by the pancreas and explains the high amino acid uptake."[3] Based on this known predilection of amino acids for the pancreas, several investigators have labeled a number of amino acids with gamma emitting nuclides and evaluated their usefulness as pancreatic scanning agents.

Beierwaltes[8] prepared ^{131}I-3-iodotyrosine(II) and determined its tissue distribution. It was found that this molecule did not selectively localize in the pancreas, presumably because it was not incorporated into the biosynthesis of the digestive enzymes. Counsell *et al.*[15] synthesized ortho-, meta-, and para-iodophenylalanines (IIIa) and radiolabeled them by halogen exchange with iodine-125. Initial tissue distribution experiments in mice

CH_2CHCO_2H / NH_2 ; X ; ^{18}F ; CH_2CHCO_2H / NH_2 ; N / H

III a. X = o,m,p-^{125}I

b. X = o,m,p-^{18}F

IV

showed very high pancreatic concentration of all three isomers; however, when evaluated in dogs, no pancreatic specificity was noted.

Wolf's group at Brookhaven National Laboratories utilized the Schiemann Reaction to prepared D,L-ortho, meta, and para-^{18}F-fluorophenylalanine (IIIb) and 5- and 6-^{18}F-fluorotryptophan (IV).[10] Uptake by the pancreas was found to be quite high and a dose dependence was noted for the localization. As the quantity of administered amino acid was increased, the pancreatic specificity decreased. Therefore, to optimize the concentration in the pancreas, high specific activity material must be used. Fluoro-18 decays by a positron emission. Collision of the positron with an electron results in annihilation of both species and the emission of two 511 keV gamma photons which can be recorded with a rectilinear scanner; therefore this nuclide is suitable for use in organ imaging radiopharmaceuticals. Unfortunately, fluorine-18 is cyclotron produced and has a half-life of only 110 min. As a result, it is only available at locations possessing a cyclotron or Van de Graaf generator.

Blau and co-workers studied a number of ^{14}C-labeled amino acids and found that about 6 per cent of the intravenously injected dose of ^{14}C-L-methionine (V) is found in the pancreas after 2 hours.[3]

$CH_3\text{-}S\text{-}CH_2CH_2CH(NH_2)\text{-}CO_2H$

V

$CH_3\text{-}{}^{75}Se\text{-}CH_2CH_2CH(NH_2)\text{-}CO_2H$

VI

Based on the chemical similarity of sulfur and selenium and the fact that selenium-75 possesses a gamma photon of 26 keV which is suitable for scanning, they prepared ^{75}Se-L-selenomethionine (VI). The radiolabeled methionine was prepared by biosynthesis -- growing yeast in a low sulfur medium enriched with high specific activity ^{75}Se as selenite -- followed by chromatographic separation of the product.[3]

In tissue distribution studies, it was found that as much as 7.6% of the administered dose (injected 30 minutes before feeding) was found in the pancreas 2 hours after injection.

Since this initial work by Blau, ^{75}Se-selenomethionine scanning has become the procedure of choice for the diagnosis of pancreatic carcinoma and pancreatitis. The procedure[16] involves injection of approximately 250 µCi (3-4 Ci/kg) of the radiopharmaceutical intravenously. Gamma camera studies are taken 10 minutes apart beginning immediately after injection. Rectilinear scanning is begun 10 minutes after injection and 30 minutes are required to complete the scan. Since the liver partially obscures the pancreas, it is desirable to obtain a ^{99m}Tc-sulfur colloid scan to allow subtraction of background radiation. Both pancreatic carcinoma and pancreatitis are characterized by diminished or absent pancreatic visualization.

Even though this procedure has increased the physician's ability to diagnose these diseases, it still suffers from some severe limitations. As was mentioned earlier, the increase in the 5 year survival rate for pancreatic cancer between 1940 and 1972 was only

0.5%. The detection of a tumor in an organ containing a neoplasm by the use of radiopharmaceuticals depends on a differential uptake (due to the kinetic differences in metabolism between tumor and surrounding benign tissue) of the radionuclide. This differential may manifest itself as a cold spot (due to decreased uptake by the malignant tissue) or as a hot spot (a result of increased uptake by the tumor). Of the two possibilities, the second is preferable since it allows for detection of tumors at an earlier stage. A radiopharmaceutical which would visualize pancreatic abnormalities as hot spots might be clearly superior to ^{75}Se-selenomethionine.

A second drawback to the use of this procedure is excessive radiation exposure to the patient. Selenium-75 has a physical half-life of 120 days and selenomethionine has a biological half-life of 20 days.[17] As a result of these excessively long half-lives, the patient is exposed to radiation for a much longer period of time than is required to perform the diagnostic scan. In addition to this excessively long exposure to radiation, selenium-75 emits a number of gamma photons which are not utilized in the scanning process (280, 136, 121, 402 and 97 keV) again exposing the patient to unnecessary radiation.

As was mentioned earlier, the anatomy of the gut organs is such that radioactivity localized in the liver partially obscures the pancreas on scintiphotos. Due to this unfavorable location of the liver, one of the most important quantities which must be

measured in evaluating a candidate pancreatic radiopharmaceutical is the ratio of activity found in the pancreas to that found in the liver (pancreas/liver). This value for ^{75}Se-selenomethionine is generally reported in the neighborhood of 3 thus necessitating the ^{99m}Tc-sulfur colloid scan of the liver. The most serious drawback, however, with respect to the detection of pancreatic carcinoma, is the fact that positive signs are found in only 25% of cases later proven by surgical exploration. Many of the problems encountered with ^{75}Se-selenomethionine could be eliminated by the development of an appropriate technetium-99m or radio-iodinated radiopharmaceutical.

D. Technetium 99m Radiopharmaceuticals

During the past 10 years, technetium-99m has become one of the most popular nuclides for use in radiopharmaceuticals. This nuclide has characteristics which very nearly conform to those described as ideal for a radiopharmaceutical in that the metastable 99m isotope decays to technetium-99 by the emission of an essentially monoenergetic gamma photon of 140 keV as shown in Figure I.[18]

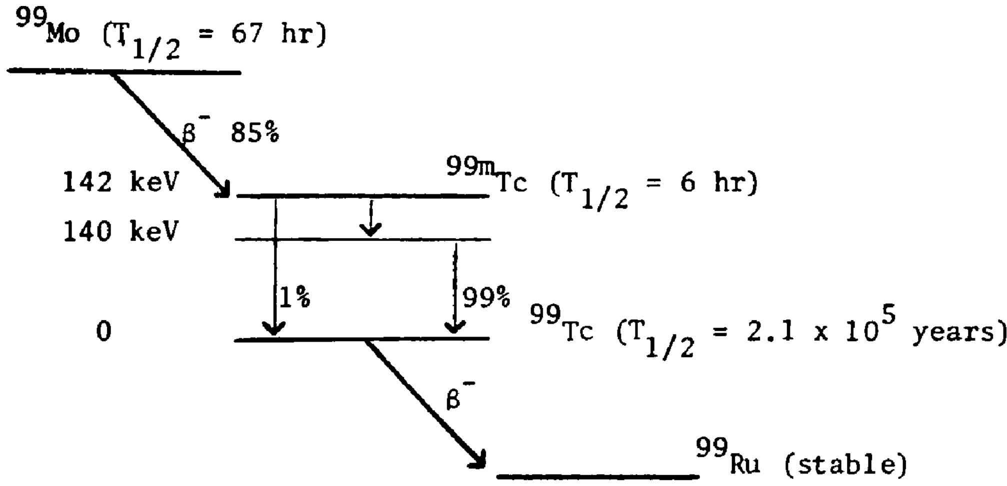

Figure I

The short (6 hour) half-life and absence of tissue destructive beta emissions allows for use of millicurie amounts of the nuclide without excessive radiation dosage to the patient.[19] The use of such high amounts results in high count rates yielding improved precision. The energy of the gamma photon is sufficient for tissue penetration yet low enough to be recorded with gamma cameras and

rectilinear scanners. Even though this nuclide decays to a radioactive product (^{99}Tc is a beta emitter) the half-life if this product is so long (2.1×10^5 years) that it results in negligible radiation dose to the patient. In addition to the desirable physical characteristics, technetium-99m is readily available to the nuclear medicine physician.

As is shown in Figure I, the parent of technetium-99m is molybdenum-99. The 67 hour half-life and the chemical properties of molybdenum make it possible for this parent-daughter system to be available in generator form. The currently available generators are based on the one developed by Richards at Brookhaven in 1957. The parent molybdenum-99 is prepared by neutron bombardment of uranium-235 or molybdenum-98. The product of these reactions is then adsorbed onto an alumina column which is enclosed in a lead shield. The daughter technetium-99m is eluted from the column with physiological saline as sodium pertechnetate ($NaTcO_4$). Peak activity is obtained if the column is eluted at 24 hour intervals. The development of this generator has made this useful isotope (^{99m}Tc) available at any qualified laboratory.

OBJECTIVE AND PROCEDURE

The objective of this research project is the development of a new pancreas imaging radiopharmaceutical to replace ^{75}Se-selenomethionine. Some of the features which would be desirable in such an agent are: (1) a pancreas to liver ratio of at least 3:1 or higher (this ratio for ^{75}Se-selenomethionine is generally reported as about 2.6:1); (2) a tumor to benign tissue ratio of greater than 1:1, preferably about 3:1 (this would result in the tumor appearing as a "hot spot" which would allow for sharper delineation of the neoplasm);and (3) incorporation of a nuclide possessing more desirable radiation characteristics than ^{75}Se. This portion of the project has dealt with the synthesis, labeling and evaluation of several candidate pancreatic radiopharmaceuticals. The compounds chosen to be labeled were based on molecules which were known to effect one of the major functions of the pancreas and, therefore, believed to localize to some extent in the pancreas. These compounds were then modified at some point remote from the "active site" in such a manner as to allow chelative attachment of the nearly ideal nuclide ^{99m}Tc or covalent bonding of ^{131}I.

RESULTS AND DISCUSSION

Hypoglycemics as Potential Radiopharmaceuticals

One of the major functions of the pancreas is the regulation of glucose metabolism by secretion of the hormone, insulin from the β-cells in the islets of Langerhans. The insulin is synthesized by and stored in the β-cells as well defined granules which can be seen in the electron micrograph.[20] These granules are transported to the cell wall and the insulin released into the blood stream in response to elevated blood glucose levels. Two classes of hypoglycemic drugs which are used in the treatment of diabetes mellitus owe their activity to an ability to stimulate the β-cells to release insulin. These classes are (1) the sulfonyl ureas such as carbutamide(VIIa), tolbutamide(VIIb) and chlorpropamide(VIIc), and (2) the sulfonamide pyrimidines, *e.g.* glycodiazine(VIII).

R_2–C$_6$H$_4$–$SO_2NHC(=O)NHR_1$

C$_6$H$_5$–SO_2–NH–(pyrimidine, N N)–$OCH_2CH_2OCH_3$

VIIa	R_1 = n-butyl	$R_2 = NH_2$
VIIb	R_1 = n-butyl	$R_2 = CH_3$
VIIc	R_1 = n-propyl	R_2 = Cl

VIII

The para-substituted sulfonylurea moiety (see general structure VII) has been found to be a common structural feature in

many compounds showing hypoglycemic activity. The structure activity studies for this class have been reviewed[21,22] and while some active agents have been discovered in which the para-substituent is varied, the most potent therapeutics appear to have a methyl group in this position. Wide variations in the nature of R_1 have been investigated with R_2 being held constant as methyl, and agents of comparable or enhanced potency compared to tolbutamide have been found.[23] Active sulfonylureas effect a marked elevation in blood insulin levels in both non-diabetic humans and in other individuals with non-insulin dependent diabetes.[24]

The fact that depancreatized humans are not susceptible to the hypoglycemic activity of tolbutamide is interpreted to imply a direct action on the pancreas.[25] This view is further supported by the observed degranulation of the pancreatic-β-cells of test animals post-dosing with sulfonyl ureas.[26] A second mechanism, activation of an inactive form of circulating insulin, has been proposed to account for the hypoglycemic action of the sulfonylureas. However, due to the observed degranulation of the β-cells, it is generally agreed that the sulfonyl ureas exert their major effects directly on the pancreas.[21,22]

Carbutamide Analogues

Initial work on this project was directed toward the preparation of a carbutamide analogue which would be capable of chelating 99m-technetium in one of its lower oxidation states. The targets aimed for were the diamino-(Xa), the guanidino-(Xb),

and the biguanidino-(Xc) analogues, all of which would be expected to form stable chelates with technetium.

$$RHN-C_6H_4-SO_2NHC(=O)NHCH_2CH_2CH_2CH_3$$

X_a $R = -CH_2CH_2N(CH_2CH_3)_2$

X_b $R = -C(=NH)-NH_2$

X_c $R = -C(=NH)-NH-C(=NH)-NH_2$

Carbutamide was prepared by reacting sulfanilamide with n-butyl isocyanate[27] or ethyl n-butylcarbamate.[28] Several attempts were made to alkylate the amino nitrogen with N,N-diethylamino-ethyl chloride. The reaction was attempted in a mixture of n-amyl alcohol and DMF 2:1 containing sodium carbonate with refluxing for 14 hours. Upon work-up, greater than 80% of unreacted carbutamide was recovered. The reaction was repeated in pure DMF which was heated at 140°C for 24 hrs. Again, only unreacted starting material was obtained.

In an attempt to prepare the guanidine compound (Xb), carbutamide was treated with ethyl isothiuronium hydrobromide in either water or ethanol. In both cases, the carbutamide was recovered unchanged.

The standard method for preparing arylbiguanides involves reacting the appropriate amine with cyanoguanidine (Scheme I) in refluxing pyridine containing pyridine hydrochloride.[29] When

$$Ar\text{-}NH_2 + N{\equiv}C\text{-}NH\text{-}\underset{\underset{NH}{\|}}{C}\text{-}NH_2 \xrightarrow[\text{pyridine}]{\text{pyridinium}^{+}\ Cl^{-}} Ar\text{-}NH\text{-}\underset{\underset{NH}{\|}}{C}\text{-}NH\text{-}\underset{\underset{NH_2^{+}}{\|}}{C}\text{-}NH_2\ Cl^{-}$$

Scheme I

carbutamide was treated with cyanoguanidine in reagent grade pyridine, sulfanilamide was obtained in a 61% yield. The reaction was repeated in anhydrous pyridine, upon work-up, 67% of the carbutamide was recovered unchanged.

The lack of reactivity of this para-amino function in the syntheses attempted above might be explained by the observation that the ring-bonded NH_2 is really a vinylogous sulfonamide, conjugated through the ring with the highly electron withdrawing sulfonyl group. Conditions sufficiently drastic to effect alkylation or substitution upon the amino group, if indeed such reactions could

be performed at all, would probably result in scission of the urea portion as observed in one case herein.

In another approach to preparing a candidate radiopharmaceutical from carbutamide, direct electrophilic iodination by iodine monochloride was investigated. Since radioiodinated forms of ICl are commercially available, the animal evaluation group associated with this project could easily prepare a "tagged" analogue in a procedure parallel to one developed herein. It was observed that facile uptake of the iodine monochloride did not occur and that refluxing in acetic acid for four hours was necessary to affect a 42% conversion. The site of iodination was confirmed by analysis of the nmr spectrum (discussed under Experimental) and the product was isolated as its hydrochloride salt (XI).

$Cl^{-}\,{}^{+}NH_3$ — $SO_2NHC(=O)NHCH_2CH_2CH_2CH_3$ — I

XI

Several analogues of tolbutamide were prepared which were found to possess hypoglycemic activity. Compounds XIa, XIb and XIc were prepared by the controlled addition of p-toluenesulfonyl isocyanate to a solution of the appropriate amine

$$p\text{-}CH_3C_6H_4SO_2NHC(=O)NHR$$

XIIa R = $-CH_2CH_2NHCH_2CH_2N(CH_3)_2$

XIIb R = $-CH_2CH_2NHCH_2CH_2NH_2$

XIIc R = $-CH_2C(=O)OC_2H_5$

XIId R = $CH_2C(=O)NHNH_2$

XIIe R = $-CH_2C(=O)OH$

XIIg R = $CH_2C(=O)Cl$

in either anhydrous dioxane or tetrahydrofuran. These compounds were obtained in yields of 76, 73 and 75% respectively.

In the projected synthesis of compound XIIb from p-toluenesulfonyl isocyanate and diethylenetriamine, an alternative reaction could have occurred at the secondary amino nitrogen to form XIIf as shown in Scheme II. For a proof of structure of the product which was obtained, the compound was treated with one

$CH_3C_6H_4SO_2N{=}C{=}O$ + $(NH_2CH_2CH_2)_2NH$ —✕→ $CH_3C_6H_4SO_2NHC(O)N(CH_2CH_2NH_2)_2$

XIIf

↘

$CH_3C_6H_4SO_2NHC(O)NHCH_2CH_2NHCH_2CH_2NH_2$

XIIb

Scheme II

equivalent of phosgene in pyridine. A molecule such as XIIf would be expected to yield either an open chain urea (both of these should have a urea carbonyl at approximately 1660 cm^{-1} in their ir spectra).[30] Compound XIIb would be expected to yield a 2-imidazolidone (carbonyl should appear at approximately 1690 cm^{-1}) as shown below. The product actually obtained from this reaction did indeed show the presence of a new absorption at 1700 cm^{-1} corresponding to the

XIIb $\xrightarrow[\text{Pyridine}]{COCl_2}$ $CH_3C_6H_4SO_2NHC(O)NHCH_2CH_2N(CH_2\text{-}CH_2NHC{=}O)$

XIII

imidazolidone carbonyl of compound XIII, thus confirming the structure of the product from the isocyanate reaction as XIIb.

In an attempt to prepare the hydrazide (XIId), XIIc was refluxed with hydrazine in anhydrous ethanol for 3 hours. The result of this reaction was recovery of 69% of unreacted starting material. The acid (XIIe) was prepared in a 95% yield by saponification of the ester (XIIc). Treatment of this acid with thionyl chloride yielded the acid chloride (XIIIg) which was reacted with hydrazine to yield a white solid whose ir and nmr spectra were consistent with those of the hydrazide (XIId). An analytical sample, however, could not be obtained. As a result of the acidic nature of the sulfonamido NH of sulfonylureas and the basicity of the terminal nitrogen of hydrazides, XIId would most likely exist in the zwiterionic form shown below:

$$CH_3C_6H_4SO_2\overset{-}{N}C(=O)NHCH_2C(=O)NHNH_3^+$$

XIId

Combustion analyses values for this compound indicated that excess hydrazine was still retained in the sample (probably as a hydrazinium salt) which could not be removed by prolonged vacuum drying. The integrated intensity of the N-H signal in the NMR also implicated the presence of additional hydrazine. Since the solubility

$$CH_3-C_6H_4-SO_2NH\overset{O}{\overset{\|}{C}}NHCH_2CH_2Cl$$

XIV

characteristics of such a target sulfonylurea hydrazide and its hydrazinium salt are so nearly comparable, fractional crystallizations to obtain pure product were not successful.

Compound XIV was prepared in a 46% yield by reacting an ethereal solution of 2-chloroethyl isocyanate with p-toluenesulfonamide in 5N sodium hydroxide. This compound can be labeled with a gamma emitting nuclide by nucleophilic displacement of the primary chlorine atom with ^{131}I-sodium iodide in acetone. This compound has been delivered to the animal testing facilities for labeling and distribution studies.

Glycodiazine Analogues

As indicated previously, glycodiazine possesses hypoglycemic activity similar to the sulfonylureas; furthermore, it is believed to exert its influence directly on the pancreas.

Several attempts were made to prepare XVa and b which are structural models of glycodiazine. The diamine portion of these molecules

$$C_6H_5SO_2NH\text{-}C_4H_2N_2\text{-}NHCH_2CH_2NR_2$$

XVa R = $-CH_3$ XVb R = $-CH_2CH_3$

would be expected to yield a stable chelate with 99m-technetium.

Two basic synthetic approaches (Scheme III and Scheme IV) to these compounds were considered. English and co-workers have

$$\underset{\text{XVI}}{C_6H_5SO_2NH\text{-}C_4H_2N_2\text{-}I} + NH_2CH_2CH_2N(CH_3)_2 \longrightarrow \text{XVa}$$

Scheme III

reported a laborious synthesis of XVI in a 61% yield[32] by the iodination of 2-benzenesulfonamidopyrimidine with molecular iodine and mercuric acetate. Preparation of a pure product by their method would prove exceedingly difficult. They also reported the direct bromination of 2-benzenesulfonamidopyrimidine with bromine in acetic acid. Because of the attendant difficulties in English's iodination, an attempt was made to improve this synthesis by using iodine monochloride as the iodinating agent. Unfortunately, this

reagent was found to be of insufficient reactivity -- even in the presence of a catalytic amount of boron trifluoride etherate. Consequently, compound XVI was, in fact, synthesized by the procedure described by English and was obtained in a 50.1% yield.

A number of attempts were made to displace the iodine of XVI with N,N-dimethylethylenediamine. This reaction is analogous to the synthesis of glycodiazine itself, in which the sodium salt of 2-methoxyethanol is reacted with XVI in a bomb at 140°C.[31] In attempt to make XVa, 2-benzenesulfonamido-5-iodopyrimidine was reacted with N,N-dimethylethylenediamine under the following conditions: (1) refluxing with excess amine for 18 hours; (2) refluxing with excess amine in DMF for 18 hours; and (3) heating with excess amine and two equivalents of sodium hydride in a bomb at 180° for 24 hours. In each case, greater than 80% of unreacted 2-benzenesulfonamido-5-iodopyrimidine was recovered. A possible explanation for the lack of success of this reaction is found in the fact that the N,N-dimethylaminoethylamine apparently decomposes to gaseous by-products under milder conditions than those necessary to displace the ring-bound iodide atom. On heating to 180°C (in a pressure vessel) the N,N-dimethylaminoethylamine, sodium hydride and the iodopyrimidine, there was a total decomposition to gaseous products which ejected the top of the vessel, after chilling to room temperature. No liquid remained and the dry sodio salt of XVI was recovered. Evidently the dialkylaminoalkylamine cannot survive these reaction conditions.

In attempt to prepare XVII, 2-benzenesulfonamidopyrimidine was nitrated with a mixture of sulfuric acid and nitric acid.

$C_6H_5SO_2NH$–(pyrimidine)–NO_2 $\xrightarrow{H_2/Pd}$ R-NH_2 $\xrightarrow{ClCH_2CH_2N(C_2H_5)_2}$ XVb

Scheme IV

This reaction resulted in the formation of a 59.7% yield of 2(m-nitrobenzenesulfonamido)pyrimidine XVIIIa. The identify of this compound was determined by analysis of its nmr spectrum. Furthermore, the melting point (215-218°C) agreed with the reported literature value for 2-(m-nitrobenzenesulfonamido)pyrimidine (217-218°C) which was prepared by the reaction of m-nitrobenzenesulfonyl chloride with 2-aminopyrimidine.[32] Apparently, the strongly acidic medium protonates the pyrimidine ring; deactivating it toward electrophilic substitution to such an extent that nitration occurs on the deactivated benzene ring. In an attempt to circumvent this deactivating effect of the strongly acidic medium, the reaction was repeated with nitric acid in acetic anhydride. The product of this reaction was determined to be 2-hydroxy-5-nitropyrimidine (XVIIIb). Stempel and Brown have reported that

they obtained the same product by the nitration of 2-aminopyrimidine.[33] It seems that the nitration proceeds as desired followed by rapid hydrolysis of the nitro- compound to XVIIIb during work-up.

Since hydrazides and hydroxamic acids are known to form stable chelates with transition metals,[34] it was decided to synthesize compounds XIX and XX and evaluate their efficacy as radiopharmaceuticals when labeled with 99m-technetium. The synthetic route to these compounds is shown in Scheme V.

XVIIIa

XVIIIb

XXII

1) $SOCl_2$

2) EtOH

XXIII $SOCl_2$

XXIV

H_2NNH_2

XXV

NH_2-R

XIX	R = NH_2
XX	R = OH
XXI	R = H

Scheme V

English has claimed the synthesis of XXII in a 63% yield by the reaction of 2-benzenesulfoamido-5-iodopyrimidine (XVI) with cuprous cyanide in boiling synthetic quinoline. They also pointed out that the same reaction in practical quinoline gave less than a 40% yield of the desired product. In this study it has been found that the same compound can be obtained in an 88% yield if DMF is used as the solvent in place of the quinoline.

Saponification of XXII with aqueous potassium hydroxide gave a 90% yield of the acid (XXIII). An attempt to esterify XXIII by refluxing in anhydrous ethanol saturated with dry HCl returned starting material. The ester (XXIV) was subsequently obtained in an 82% yield by treating XXVIII with thionyl chloride to form the acid chloride which was refluxed in anhydrous ethanol to yield the desired product.

An attempt to prepare the hydrazide (XIX) by treating this ester (XXIV) with hydrazine hydrate in refluxing ethanol resulted in formation of the acid (XXIII) in a 67% yield. The hydrazide was successfully obtained in a 60% yield by treatment of XXIII with thionyl chloride to give the acid chloride (XXV) which was then reacted with aqueous hydrazine to yield the desired product. A similar procedure was used to prepare the amide (XXI) and the hydroxamic acid (XX).

Glucose Tolerance Tests

To determine if these tolbutamide and glycodiazine analogues possessed hypoglycemic activity (and thus pancreatotrophic

character) they were submitted to McNeil Laboratories for screening in the glucose tolerance test. The compounds tested are shown in Table II. The candidate agents were administered as single *i.p.* injections at 50 mg/kg in Methocel to three Sprague-Dawley rats fasted for 24 hours predosing. The test animals and four controls were glucose loaded with 1g/kg given orally and blood

General structure: 4-CH_3-C_6H_4-$SO_2NHC(=O)NH-R$ — hypoglycemic activity a+50.

	R	
XIIa	$-CH_2CH_2NHCH_2CH_2N(CH_3)_2$	Active
XIIb	$-CH_2CH_2NHCH_2CH_2NH_2$	Slightly Active
XIIc	$-CH_2C(=O)-OC_2H_3$	Inactive
XIIe	$-CH_2C(=O)-OH$	Hyperglycemic

General structure: C_6H_5-SO_2NH-(2-pyrimidinyl)-R

	R	
XXIII	$-CO_2H$	Inactive
XXIV	$-CO_2C_2H_5$	Inactive
XIX	$CONHNH_2$	Slightly active at 50 mg/kg

Table II

aliquots were withdrawn at 30 minute intervals and analyzed for mg glucose/100 ml of blood. Compound XIIa displayed marked hypoglycemic effects and dropped blood glucose levels by 34 ± 6 mg at 30 minutes and 15 ± 1 mg at 60 minutes compared to standards --

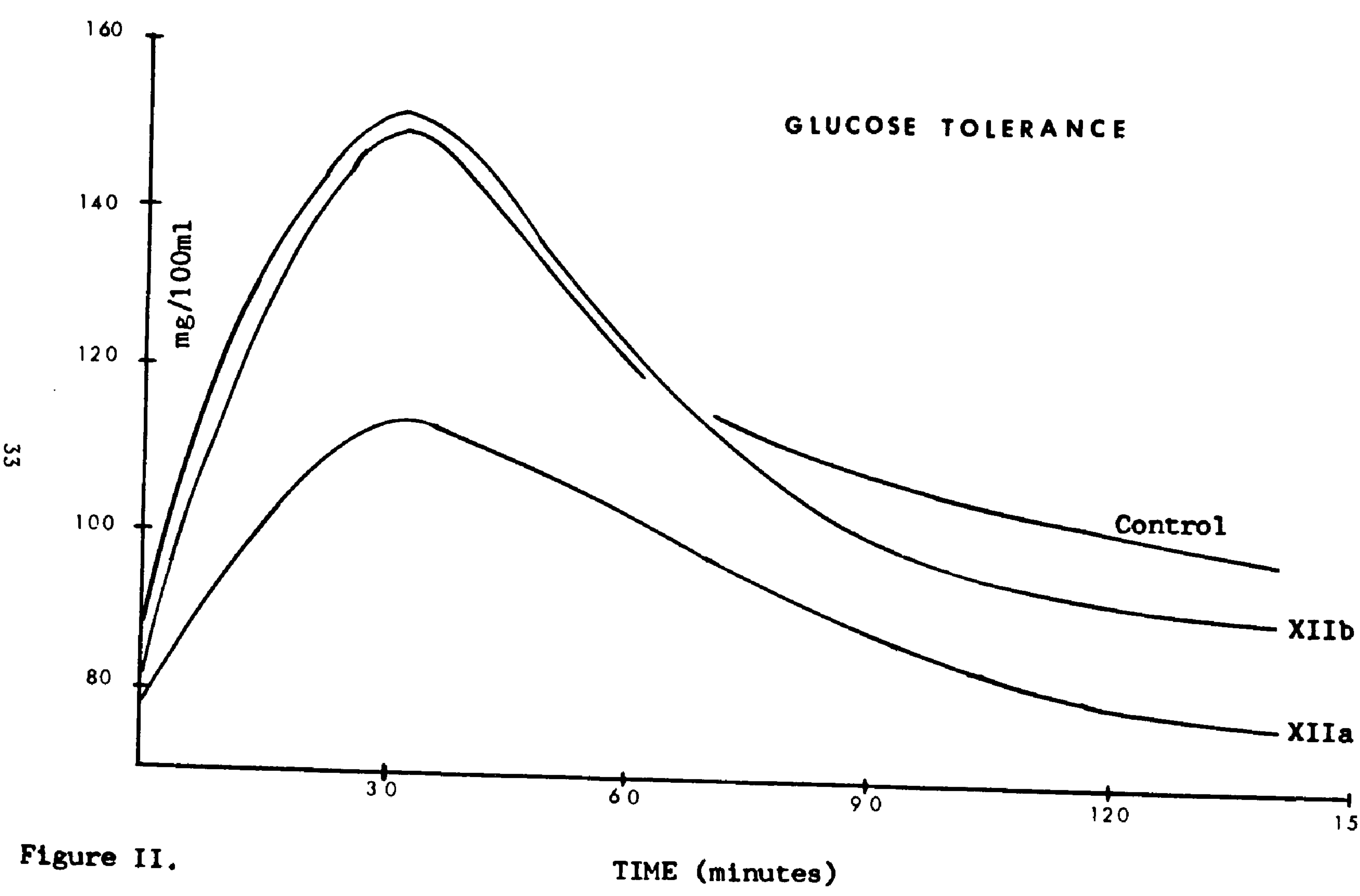

Figure II.

a hypoglycemic potency approximately equal to that of tolbutamide. Compound XIIb showed slight activity at 90 minutes. A slight hyperglycemic effect was detected for XIIe while XIIc showed no activity at all. Of the glycodiazine analogues, only XXIa displayed activity, a modest hypoglycemia at an elevated dose of 100 mg/kg, while the others were inactive. Figure II compares the glucose tolerance tests for XIIa and XIIb with that of controls. Based on the results of these tests, compounds XIIa and XIIb were chosen for labeling with 99m-technetium and distribution studies in rats.

<u>99m-Technetium Chelates</u>

The ^{99m}technetium required for these studies was obtained from a Squibb Technetope Generator. Elution of this generator with physiological saline (future references to saline shall be taken to mean physiological saline, 0.8% sodium chloride in distilled water) yields an aqueous solution of sodium pertechnetate ($NaTcO_4$). Prior to chelate formation, it is necessary to reduce the pertechnetate ion to a lower oxidation state. When this project began, there were a number of reductions of this type available in the literature. Eckelman and co-workers have reported that a ^{99m}Tc-DTPA* chelate can be prepared in good yield by reducing pertechnetate ion (oxidation state of +7) to TcO^{+2}

---

* DTPA is a common abbreviation for diethylenetriamine pentaacetic acid.

(oxidation state of +4) with ferrous ion in the presence of DTPA and ascorbic acid.[35] Three ^{99m}Tc species are present in the "reduced" solution; $^{99m}TcO^{+2}$ bound to DTA, TcO^{+2} unbound, in the form of TcO_2, and unreduced $^{99m}TcO_4^-$. By the use of gel chromatography, the authors were able to obtain the chelate in a 95% yield free of contamination by pertechnetate or reduced unbound technetium. The chelate was eluted from the column in an elution volume of 12-20 ml, pertechnetate was eluted between 38 and 46 ml and the reduced unbound technetium was irreversibly bound to the column. Of the initial radioactivity, 95% was recovered in the chelate fraction, 1% in the pertechnetate fraction and the remaining 4% was retained by the column. Paper chromatography on Whatman paper eluted with saline gave three radioactive spots, $R_f = 0$, (hydrolyzed reduced technetium), $R_f = 0.65\text{-}0.80$ (TcO_4^-) and R_f 0.85-0.95 (chelate).[36]

In this study the identical procedure was employed to prepare 99m-technetium chelates of compounds XIIa and XIIb. After the reaction (reduction and chelation) was complete, the solution was purified by elution from a Sephadex G-25-m column with saline. A typical elution curve for this procedure is shown in Figure III. As can be seen in Figure III the chelate is eluted between 16 and 37 ml and pertechnetate between 38 and 64 ml (as expected from the precedent of Eckelman's work). The composition of the fraction between 38 and 64 ml was confirmed by chromatographing a solution containing only pertechnetate. It is apparent from Figure III

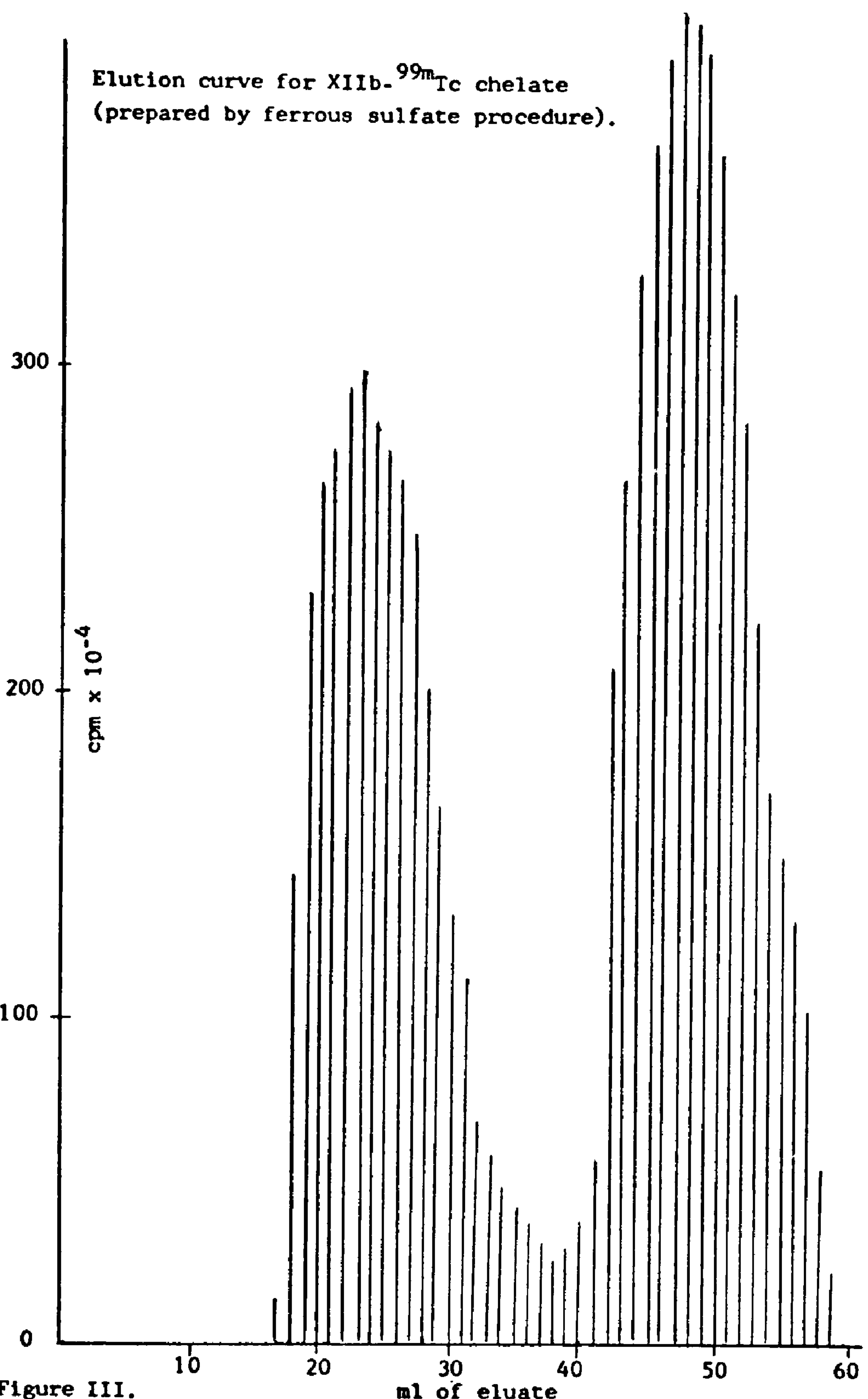

Figure III.

Table III. Data from purification XIIb. ^{99m}Tc Chelate (prepared by ferrous sulfate-ascorbic acid procedure) on Sephadex G-25-m Column

Fraction	Cpm x 10^{-4}	Fraction	Cpm x 10^{-4}	Fraction	Cpm x 10^{-4}	Fraction	Cpm x 10^{-4}
1-15	0	29	66	44	265	58	75
16	1	30	131	45	330	59	54
17	12	31	112	46	369	60	45
18	144	32	69	47	395	61	33
19	232	33	58	48	411	62	25
20	264	34	49	49	405	63	16
21	275	35	41	50	395	64	9
22	294	36	36	51	365		
23	298	37	30	52	321		
24	282	38	27	53	283		
25	273	39	29	54	221		
26	264	40	38	55	136		
27	218	41	58	56	128		
28	200	42-43	207	57	102		

Total for chelate fraction (16-37 ml) = 3449

Total for TcO_4^- fraction (38-64 ml) = 4742

TcO_4^-/chelate ≡ 1.4

that the reduction was inefficient in this case. Table III shows the actual value of the radioactivity in each fraction. The ratio of activity found in the pertechnetate fraction to that in the chelate fraction was approximately 1.4.

Paper chromatography of the crude chelate solution on Whatman number 1 eluted with saline gave three radioactive spots: origin, reduced hydrolyzed technetium (TcO_2); R_f = 0.55-0.8, TcO_4^- and 0.8 to 1.0, Tc chelate. Table IV shows the breakdown of the chromatogram for XIIb. This chromatogram indicates that the crude reaction mixture consists of 31% reduced hydrolyzed technetium; 46% pertechnetate and 23% chelate; thus a pertechnetate to chelate ratio of approximately 2. Since it was not always possible to separate the pertechnetate and chelate spots, a second system was utilized. Thin layer chromatography on Baker-flex silica gel-1B eluted with 85% methanol gave two spots. Pertechnetate migrated with an R_f of 0.7 to 1.0 while both the chelate and reduced hydrolyzed technetium remained at the origin. Therefore, by using both chromatography procedures, a discrete spot can be obtained for both reduced hydrolyzed technetium and pertechnetate, the remaining radioactivity being due to the chelate. Table V shows a typical silica gel chromatogram of the chelate fraction after purification by gel filtration. This chromatogram shows that 96% of the radioactivity in the chelate fraction is attributable to the ^{99m}Tc-chelate. No reduced hydrolyzed technetium is present at the origin, since it is irreversibly bound to

Table IV

Chromatography results of crude chelate solution on Whatman paper #1 eluted with saline

	TcO^{+2}	TcO_4^-	Chelate	
R_f	0-0.49	0.5-0.79	0.8-1.0	
Cpm-Background	84114	133 784	64684	282582
%	30	47	23	

TcO_4^-/Chelate 2

Table V

Chromatography results on purified chelate solution on Baker-flex silica gel-113 eluted with 85% methanol

	$TcO^{+2}+TcO_4^-$		Chelate
R_f	0-0.33	0.33-0.66	0.66-0.9
Cpm-Background	8655	82	267
%	96	1	3

the column. The small amount of pertechnetate present in the chelate fraction is probably a result of air oxidation of the chelated reduced technetium. Several attempts to increase the amount of reduced chelated technetium obtained by extended reaction times and increased quantities of ferrous sulfate yielded similar results no improvement in chelation percentage.

Eckelman and co-workers also reported that the technetium-DTPA chelate could be prepared in a 97% yield by using stannous chloride as the reducing agent.[35] In this case, they claimed that no unreduced pertechnetate could be detected. In this work, the $SnCl_2$ procedure was evaluated using gastrin tetrapeptide as a ligand (the reason for the choice of this compound will be discussed later). The gel chromatography results and TLC results indicated that indeed no 99m-technetium in the form of pertechnetate was present.

Because of the greater reducing efficiency of stannous chloride, the labeling and animal distribution studies for compounds XIIa and XIIb were repeated.[36] A typical elution curve for these compounds is shown in Figure IV.

Animal Distribution Studies

In initial animal distribution studies performed in this project, a rather elevated experimental variation from animal-to-animal in the same dosage administration was observed. Percent dose per gram values often varied by 100 percent from run-to-run under comparable experimental conditions. Initial studies were

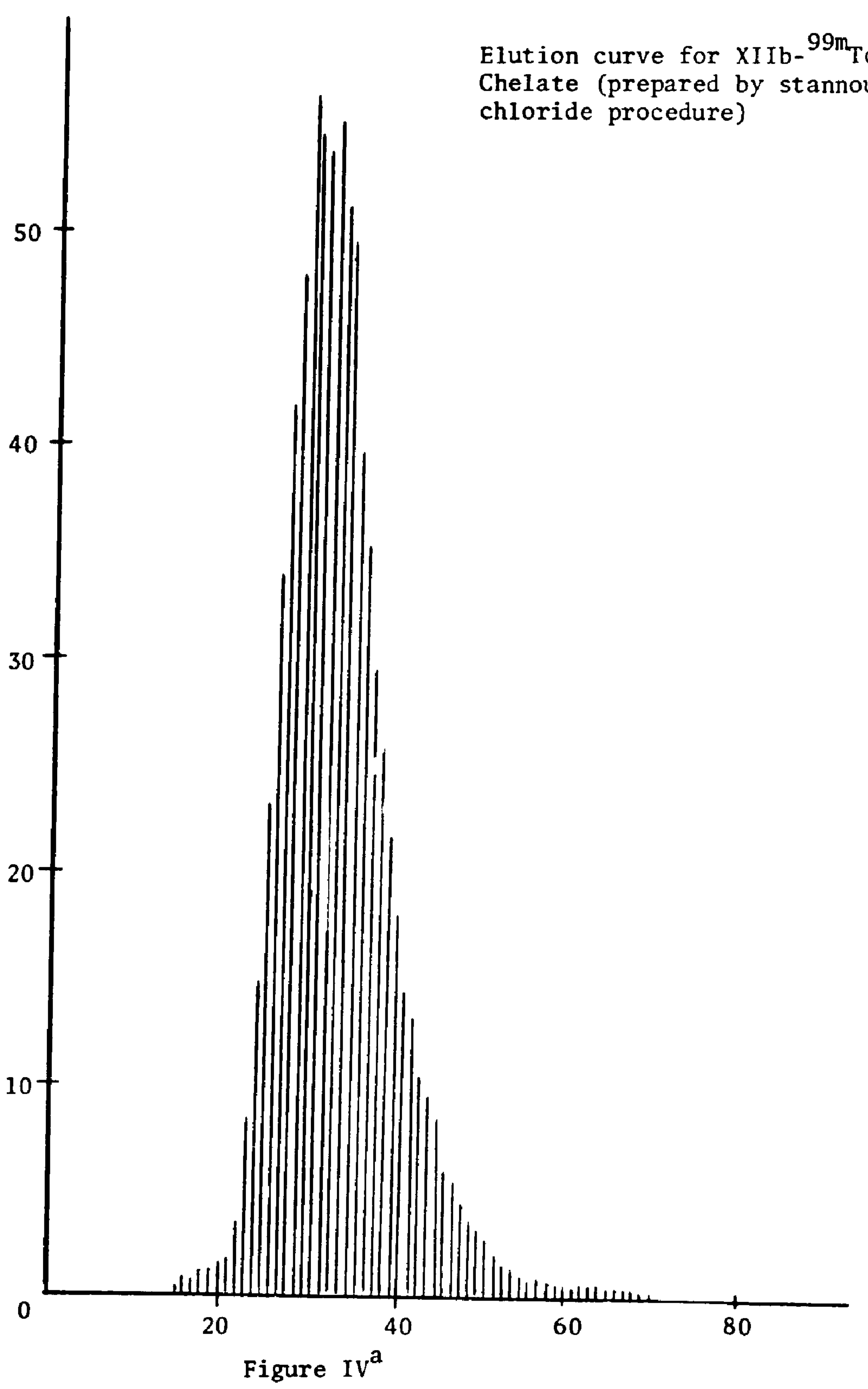

Figure IV[a]

a. See reference 36

performed in an outbred strain of albino Wistar rats (see experimental section for details) with no preference for sex or weight in the choice of the particular experimental animal. After a number of experiments, it was determined that some correction for animal weight was necessary since it is obvious that the organ distribution data will be profoundly affected by variance in the size of rat used due to blood volume dilution effects. However, current publications on organ distribution studies of this type do not appear to take such animal size effects into account. Either no comment is made on reproducibility of measurements or extremely large standard deviations are reported.

Two methods of correcting for dilution effects were used. The best internal agreement of the experimental data was found to be in a normalization of variable body weights to a standard weight. When the body weight was not known, adequate data could be obtained by normalizing the variable rat kidney weights to a standard kidney weight. The values for individual organ concentrations were determined (see experimental section for details) in per cent dose per gram. These values were then normalized to a standard 200g rat by multiplying by N or to a standard size kidney of 1g by multiplying by N' where N and N' are defined as:

$$N = \text{weight of animal}/200\ g$$

$$N' = \text{weight of animal kidney}/1\ g$$

Table VI. XIIa ^{99m}Tc chelate (prepared by ferrous sulfate procedure normal animals, 2 rats per point)

	15 min*	30 min	60 min
Lung	0.376±0.004	0.269±0.028	0.175±0.012
Liver	0.292±0.008	0.300±0.025	0.207±0.016
Spleen	0.160±0.007	0.097±0.019	0.081±0.002
Kidney	1.84 ±0.40	1.65 ±0.25	2.03 ±0.07
Pancreas	0.136±0.002	0.102±0.013	0.068±0.009
Blood	0.443	0.265±0.035	0.172±0.017
Pancreas/Liver	0.5	0.34	0.32

* Three rats

Table VII. XIIb ^{99m}Tc Chelate (Prepared by Ferrous Sulfate Procedure, Normal Animals, 2 rats per point)

	15 min	30 min	60 min
Lung	0.296±0.043*	0.226±0.031	0.169±0.020
Liver	0.158±0.031*	0.490±0.013	0.503±0.007
Spleen	0.78±0.11	0.442±0.044	0.194±0.062
Kidney	3.70±0.97*	4.36±0.018	2.44±0.040
Pancreas	0.150±0.070*	0.107±0.004	0.046±0.003
Blood	0.320±0.012	0.366±0.030	0.108
Pancreas/Liver	0.95	0.22	0.09

* Three rats

Table VIII[a]

XIIa-^{99m}Tc chelate tissue distribution

1. Normal animals

	15 minutes[ƒ]	30 minutes[ƒ]	60 minutes[ƒ]
Lung	0.954 ± 0.111	0.665 ± 0.070	0.474 ± 0.009
Liver	0.916 ± 0.009	0.797 ± 0.081	0.665 ± 0.028
Spleen	0.426 ± 0.023	0.323 ± 0.023	0.237 ± 0.010
Kidney	5.402 ± 0.067	6.940 ± 0.879	6.779 ± 0.017
Pancreas	0.309 ± 0.012	0.265 ± 0.034	0.173 ± 0.007
Blood	1.165 ± 0.060	0.859 ± 0.037	0.600 ± 0.074
Pancreas/Liver	0.337	0.332	0.260

ƒ Two rats

2. Predosed with Imferon

	15 minutes[ƒ]	30 minutes[ƒƒ]	60 minutes[ƒ]
Lung	1.255 ± 0.336	0.878 ± 0.209	0.675 ± 0.162
Liver	1.356 ± 0.248	1.304 ± 0.109	1.185 ± 0.249
Spleen	0.492 ± 0.141	0.397 ± 0.056	0.392 ± 0.023
Kidney	7.528 ± 1.786	7.021 ± 4.922	8.309 ± 1.116
Pancreas	0.514 ± 0.108	0.438 ± 0.059	0.320 ± 0.117
Blood	1.200 ± 0.189	0.798 ± 0.192	0.656 ± 0.102
Pancreas/Liver	0.379	0.336	0.270

ƒ Three rats
ƒƒ Five rats
a See Reference 36.

Table VIII (Continued)

3. Dosed with morphine

	15 minutes	30 minutes	60 minutes
Lung	1.171 ± 0.312	0.786 ± 0.127	0.538 ± 0.046
Liver	1.496 ± 0.248	1.058 ± 0.224	0.949 ± 0.379
Spleen	0.625 ± 0.598	0.412 ± 0.115	0.303 ± 0.055
Kidney	5.129 ± 0.598	4.789 ± 1.161	5.031 ± 0.898
Pancreas	0.642 ± 0.146	0.425 ± 0.112	0.288 ± 0.019
Blood	1.580 ± 0.234	0.963 ± 0.086	0.735 ± 0.064
Pancreas/Liver	0.429	0.402	0.303

4. Dosed with Lasix

	15 minutes	30 minutes	60 minutes
Lung	1.327 ± 0.625	0.743 ± 0.172	0.533 ± 0.156
Liver	1.188 ± 0.263	1.064 ± 0.143	0.798 ± 0.109
Spleen	0.536 ± 0.153	0.330 ± 0.034	0.217 ± 0.038
Kidney	5.727 ± 2.119	5.248 ± 1.550	6.516 ± 3.637
Pancreas	0.460 ± 0.040	0.390 ± 0.047	0.234 ± 0.046
Blood	1.387 ± 0.140	0.887 ± 0.187	0.541 ± 0.120
Pancreas/Liver	0.387	0.367	0.293

Table VIII (Continued)

5. Predosed with Ca-EDTA

	15 minutes	30 minutes	60 minutes
Lung	0.788 ± 0.114	0.456 ± 0.112	0.296 ± 0.070
Liver	0.991 ± 0.173	0.748 ± 0.135	0.646 ± 0.146
Spleen	0.325 ± 0.151	0.260 ± 0.065	0.185 ± 0.032
Kidney	4.798 ± 0.845	4.850 ± 1.515	4.763 ± 0.175
Pancreas	0.474 ± 0.124	0.341 ± 0.071	0.199 ± 0.035
Blood	1.050 ± 0.111	0.612 ± 0.090	0.372 ± 0.094
Pancreas/Liver	0.478	0.456	0.308

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

Table IX[a]

XIIb-^{99m}Tc chelate tissue distribution

1. Normal animals

	15 minutes[ƒ]	30 minutes[ƒ]	60 minutes[ƒ]
Lung	0.759 ± 0.025	0.560 ± 0.057	0.358 ± 0.013
Liver	0.837 ± 0.003	0.690 ± 0.001	0.603 ± 0.001
Spleen	0.304 ± 0.006	0.232 ± 0.003	0.155 ± 0.001
Kidney	6.654 ± 0.202	5.907 ± 0.482	6.142 ± 0.013
Pancreas	0.312 ± 0.011	0.199 ± 0.003	0.156 ± 0.010
Blood	0.968 ± 0.002	0.683 ± 0.103	0.409 ± 0.010
Pancreas/Liver	0.373	0.288	0.259

2. Predosed with Imferon

	15 minutes	30 minutes	60 minutes
Lung	0.875 ± 0.359	0.664 ± 0.285	0.479 ± 0.053
Liver	0.709 ± 0.268	0.814 ± 0.275	0.656 ± 0.280
Spleen	0.331 ± 0.164	0.367 ± 0.225	0.184 ± 0.056
Kidney	5.449 ± 2.826	8.369 ± 4.376	10.145 ± 9.998
Pancreas	0.271 ± 0.079	0.242 ± 0.096	0.141 ± 0.005
Blood	0.898 ± 0.435	0.706 ± 0.352	0.451 ± 0.169
Pancreas/Liver	0.382	0.297	0.215

ƒ Two rats

a See Reference 36.

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

Table IX (Continued

3. Dosed with morphine

	15 minutes	30 minutes	60 minutes ⸸
Lung	0.812 ± 0.212	0.528 ± 0.151	0.393 ± 0.068
Liver	0.746 ± 0.271	0.432 ± 0.077	0.350 ± 0.010
Spleen	0.332 ± 0.136	0.212 ± 0.028	0.199 ± 0.042
Kidney	5.631 ± 0.635	6.008 ± 0.972	6.239 ± 0.197
Pancreas	0.334 ± 0.104	0.265 ± 0.100	0.193 ± 0.025
Blood	0.979 ± 0.401	0.555 ± 0.140	0.352 ± 0.073
Pancreas/Liver	0.448	0.613	0.551

⸸ Two rats

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

Table X [a]

^{99m}Tc (reduced) tissue distribution

	15 minutes ≠	30 minutes ≠	60 minutes ≠
Lung	1.192 ± 0.142	1.072 ± 0.008	0.910 ± 0.192
Liver	6.595 ± 0.964	6.078 ± 1.600	6.114 ± 0.805
Spleen	2.438 ± 0.144	4.123 ± 0.841	2.202 ± 1.194
Kidney	6.694 ± 1.402	6.397 ± 0.815	7.179 ± 0.240
Pancreas	0.484 ± 0.368	0.497 ± 0.121	0.410 ± 0.143
Blood	2.639 ± 0.004	1.907 ± 0.073	1.569 ± 0.235
Pancreas/liver	0.073	0.082	0.067

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

≠ Two rats

a See Reference 36.

In subsequent tissue distribution studies, it was found that a greater reproducibility of experimental values could be obtained if only male rats of the inbred albino Fisher strain were used. These values were also normalized to a standard 200 g rat as described above.

Tables VI through IX show the results of animal distribution studies for compounds XIIa and XIIb. The chelates described in Tables VI and VII were prepared by the ferrous sulfate-ascorbic acid procedure while those in Tables VIII and IX were prepared by the stannous chloride procedure. The differences in distribution of compounds labeled by the ferrous ion vs. the stannous ion reduction may simply reflect a dose dependence for the organ distributions of these chelates. The amount of radiopharmaceutical injected when the chelate was prepared by the ferrous ion procedure was considerably lower than that when the chelate was prepared by the stannous ion procedure. Atkins and co-workers have reported a similar phenomenon in the tissue distribution of ^{18}F-6-fluorotryptophan.[37]

The organ of highest concentration for each of these agents was the kidney at each of the times studied. This was found to be the case regardless of the labeling procedure used. Several authors have suggested that chelates are not specific for any particular organ but are cleared from the blood into the kidney by glomerular filtration.[38] However, a number of ^{99m}Tc-chelates have recently been developed for imaging loci other than the kidney: the gall bladder,[39] the liver,[40] amebic abscesses,[41] and myocardial infarcts.[42] These results indicate that such chelates may, in some cases, retain the tissue specificity of the parent ligand.

The critical pancreas/liver ratio in no case approached the desired value of 3:1. This ratio generally did not vary significantly from a value of 0.3:1 throughout the time of the study, with no significant incorporation of the agent into the pancreas, since the blood in all cases retained a higher concentration than the pancreas.

Basically, there are only two ways to increase the pancreas to liver ratio: (1) increase the concentration in the pancreas, or (2) decrease the concentration in the liver. One possible cause for the elevated liver uptake is the *in vivo* dissociation of the chelate to yield free $^{99m}TcO^{+2}$. This reduced form of technetium has been reported to bind to blood protein and localize to a great extent in the liver.[43] In this study, this predilection for the liver was confirmed by determining the organ distribution for reduced hydrolyzed technetium in the absence of

any ligand. The results of this determination are shown in Table X.

In order to determine if the chelate was, in fact exchanging its label to blood protein, the test animals were predosed with a soluble iron-dextran complex (Imferon, Lakeside Laboratories) to saturate the binding sites of the blood proteins. The results of this study are shown in Table VIII-2. The effect of this procedure on the pancreas to liver ratio was negligible.

Similarly, predosing 60 minutes prior to administration of the ^{99m}Tc-chelate of XXIa with a Ca EDTA* chelate (to circumvent the possibility that indigenous metal ions in the blood might exchange with the cationic technetium nuclide and thus promote dissociation of the radiopharmaceutical) did not substantially alter the organ distribution.

In attempt to prevent the rapid kidney incorporation of these tagged chelates, an injectable diuretic furosemide (Lasix, Hoechst Pharmaceutical Co.) was administered simultaneously with the radiopharmaceutical. In theory, this stimulation to rapid urination might be expected to lower the kidney uptake by the labeled chelate. There was, however, no significant effect on either the pancreas/liver ratio or on the rate of kidney uptake.

The final attempt to enhance the pancreas/liver ratio involved coadministration of morphine sulfate, often utilized in ^{75}Se-selenomethionine studies as an anticholinergic to enhance pancreas levels.[44,45] It is believed that morphine sulfate

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* EDTA is the abbreviation for ethylenediamine tetraacetic acid.

constricts the outlet duct of pancreas and retards the flow of pancreatic juices into the intestine. Even the treatment of the test animals with morphine sulfate, however, did not alter the pancreas/liver ratio for compound XIIa. However, this key ratio experienced a marked increase to 0.6/1 for compound XIIb (see Tables VIII-3 and IX-3).

The low pancreatic uptake and low pancreas/liver ratios for these chelates may be accounted for by three possible explanations. First, it may simply reflect the fact that the β-cells (the assumed site of action of the sulfonylureas) constitute only 1-4% by weight of the pancreas.[46] Second, the formation of a chelate (a significant electronic modification) may have destroyed the intial pancreatotrophic character of the compound. Finally, the original assumption that these hypoglycemic compounds concentrate in the pancreas may be in error.

If the first explanation is, indeed, the proper explanation, future work may prove fruitful. Since this study is primarily concerned with detection of pancreatic neoplasms, it should be noted that in β-cell adenocarcinomas (a common type of pancreatic cancer) the β-cells often constitute up to 50% of the weight of the pancreas.[46] It is possible, therefore, that these agents may prove effective in the detection of this type of cancer and will, in fact, be evaluated for this purpose at a later date.

A report by Beierwaltes and co-workers[47] on the tissue distribution of ^{125}I-iodopropamide (XXVa) - a bioisostere of the

European hypoglycemic chlorpropamide (XXVb) - seems to lend support

$$X\text{-}C_6H_4\text{-}SO_2NHC(=O)NHCH_2CH_2Cl$$

XXVa $X = {}^{125}I$-

XXVb $X = Cl$-

to the third explanation, since pancreatic uptake was quite low. Although no glucose tolerance test results were reported, they did state that the electron-micrograph showed degranulation of the β-cells. When glucose tolerance test and organ distribution results are completed, by other workers, on the radioiodinated hypoglycemics prepared herein a more definitive answer to this question will be available.

$$NH_2(I^{131})C_6H_3\text{-}SO_2NHC(=O)NHCH_2CH_2CH_2CH_3$$

$$CH_3\text{-}C_6H_4\text{-}SO_2NHC(=O)NHCH_2CH_2I^{131}$$

Gastrin Tetrapeptide

Gastrin is a polypeptide hormone which is produced by the pyloric mucosa, apparently stimulated to do so by substances present in or derived from food.[48] Intravenous injection of this hormone causes a rapid increase in the volume-flow and enzyme output of the pancreas.[49] "The entire range of physiological activities displayed by natural gastrin are possessed by the C-terminal tetrapeptide sequence Try-Met-Asp-Phe-NH_2"[49] [gastrin tetrapeptide (XXVI)].

Diamine

XXVI

Since hormones are by definition "substances manufactured by cells in minute amounts which produce characteristic physiological effects upon other cells, usually in some 'target' organ or tissue remote from the source of the hormone,"[51] it appears that this compound might be an ideal candidate for use as a pancreatotrophic radiopharmaceutical.

From a structural viewpoint, the "diamino" tryptophan portion of this polypeptide might form a stable chelate with reduced ^{99m}technetium. The stannous chloride labeling procedure yielded a solution which was shown by paper chromatography to consist of approximately 47% chelate and 53% reduced hydrolyzed technetium. Both thin layer and gel filtration chromatography indicated that no technetium in the form of pertechnetate ion was present.

Table XI. ^{99m}Tc-Gastrin tetrapeptide chelate.

	% Dose/g 15 min*	% Dose/g 30 min*	% Dose/g 60 min*
Lung	1.06 ± 0.07	0.90 ± 0.15	0.844 ± 0.008
Liver	0.573 ± 0.08	0.53 ± 0.10	0.634 ± 0.002
Spleen	0.333 ± 0.049	0.247 ± 0.031	0.168 ± 0.070
Kidney	2.74 ± 0.015	2.06 ± 0.08	2.18 ± 0.16
Pancreas	0.440 ± 0.048	0.354 ± 0.010	0.286 ± 0.014
Stomach	0.53 ± 0.13	0.574 ± 0.092	0.432 ± 0.037
Blood	1.31 ± 0.01	0.98 ± 0.19	1.05 ± 0.67
Pancreas/Liver	0.768	0.668	0.451

* Average of 2 rats

After purification on a Sephadex column, the tissue distribution for this chelate was determined. The results of this study are shown in Table XI. Again, the greatest concentration of the radionuclide is in the kidney. In this case, the pancreas to liver ratio is about double that obtained for the tolbutamide analogue chelates.

Streptozotocin Analogues

Streptozotocin (XXVIII) is a 2-deoxy-D-glucose derivative of N-methyl-N-nitrosourea which was first isolated from *Streptomyces acromogenes* by investigators at Upjohn Research Laboratories.[52,53] This compound was found to have both Gram positive and Gram negative antibacterial activity.[52,53] During the early *in vivo* studies,

XXVIII

this broad spectrum antibiotic was found to be cytotoxic to the insulin producing β-cells of the pancreas,[55] resulting in the production of frank diabetes at elevated dose levels. As a result of this diabetogenic activity, streptozotocin is currently used by pharmacologists to produce a permanent state of diabetes in experimental animals (which are used for the evaluation of potential hypoglycemic compounds).

During initial biological screening, it was also observed that this compound was able to inhibit the growth of a number of animal tumors such as L5178Y, Ehrlich's carcinoma, the Walter 256 carcinosarcoma,[56] and, in addition, it has been used for treatment of insulinomas in man.[57]

At the present time, the exact mechanism of the cytotoxic and antitumor activity of streptozotocin has not been clearly elucidated. However, it has been shown that this compound decomposes at physiological pH to diazomethane (XXIX) and compound (XXX),[58] and it is generally believed that the highly toxic diazomethane is the source of activity.

The specific action of streptozotocin on the pancreatic β-cells coupled with its broad scope of antitumor activity makes this compound an ideal candidate for preparation of a pancreatotrophic radiopharmaceutical. Of course, a radiopharmaceutical which caused permanent diabetes would be of little use. Fortunately, the dose required for organ imaging compounds is considerably lower than the diabetogenic dose of streptozotocin. Furthermore, Schein and co-workers have established that administration of nicotinamide blocks the diabetogenicity without affecting the anticancer activity.[59]

In this study, an attempt was made to prepare a ^{99m}Tc-streptozotocin chelate (the antibiotic was obtained as a sample from Upjohn Laboratories) using the stannous chloride labeling procedure. All three chromatographic systems (paper, thin layer and gel filtration) showed that all of the radionuclide was present

in the form of reduced hydrolyzed technetium, indicating that streptozotocin is a poor ligand for cationic technetium.

XXVIII $\xrightarrow{\text{pH 7.4}}$ XXX + $\bar{C}H_2-\overset{+}{N}\equiv N:$ XXIX

Synthesis of Streptozotocin Analogues

As part of the structure proof for streptozotocin, Herr and co-workers synthesized this compound from D-2-glucosamine hydrochloride (XXXI) as shown in Scheme VI.[54]

XXXI → (O=CH–C₆H₄–OCH₃) → XXXII → XXXIII → XXXIV

1) CH_3CO_2Na

2) $CH_3\text{-}N{=}C{=}O$

XXXV

XXVIII

Scheme VI

The amino nitrogen was first protected by reaction with p-anisaldehyde to form the imine (XXXII). Acetylation of XXXII with acetic anhydride in pyridine yielded XXXIII which was hydrolyzed to 2-tetra-O-acetyl glucosamine hydrochloride (XXXIV). Treatment of XXXIV with methyl isocyanate yielded the urea (XXXV)

which was nitrosated with nitrosyl chloride in pyridine. The final product was obtained by deacetylation of XXXVII with methanolic ammonia at -10°C and was shown to be identical in all respects with an authentic sample of streptozotocin isolated from <u>Streptomyces achromogenes</u>.

The experimental procedure for the synthesis of 2-tetra-O-acetyl-D-glucosamine hydrochloride had previously been reported in the literature[60]; however, Herr and co-workers did not give any details or yields for the remaining steps in this sequence.

As part of this project, it was decided to synthesize the 2-chloroethyl analogue (XXXIXa) of streptozotocin. A very logical candidate radiopharmaceutical based on streptozotocin would be the 2-chloroethyl analogue (XXXIXa) shown below. Not only would this compound possess the basic structural nucleus of the parent streptozotocin but it would also carry the typical alkylating nitrogen-mustard side chain so often found in anticancer drugs. In addition, the combination of the urea moiety, with its N-nitroso and N-chloroethyl sidechains, is a close structural model to the highly potent antitumor agent CCNU (XXXIXc). This compound would

XXXIXa X = Cl

b X = ^{131}I

XXXIXc

XXXIV

$O=C=NCH_2CH_2Cl$

XL

$NaNO_2/HCl$

AcOH

XLIII

XLI

$O=C=N-CH_2CH_2Cl$

Ac_2O/pyridine

XXXI

XXXIXa

Scheme VII

be expected to undergo facile halogen exchange with ^{131}I-sodium iodide to yield XXXIXb which could be evaluated as a pancreatotrophic radiopharmaceutical.

Compound XXXIV was prepared from D-2-glucosamine as shown in Scheme VI, in an overall yield of 57%. An aqueous solution of this salt was neutralized with sodium acetate, followed by extraction of the free base into chloroform. Treatment of the amine with 2-chloroethyl isocyanate yielded XL in 75% yield as shown in Scheme VII. Nitrosation of XL with sodium nitrite/HCl in acetic acid resulted in the formation of XLI in a 63% yield. That the nitrosation had indeed occurred on the nitrogen shown was confirmed by analysis of the nmr spectra of XL and XLI (This point will be discussed in greater detail in a later section.).

An attempt was made to deacetylate XLI with methanolic ammonia at -10°C. This reaction resulted in the formation of a viscous oil which could not be characterized. Hessler and Jahnke have reported that the corresponding deacetylation step in the original synthesis of streptozotocin was accompanied by severe degradation.[61] Deacetylation of XLI is further complicated by the presence of the 2-chloroethyl group which can lead to additional side reactions such as the displacement of the primary chlorine atom by ammonia or formation of the oxazole (XLII).[62]

XLII

The reason for the preparation of the acetylated compounds initially was to preclude the possibility of carbamate formation by reaction of the isocyanate with the sugar hydroxyls. Although the acetyl groups could not be successfully removed to yield the desired target (XXXIXa), these acetyl compounds were found to be of more pharmacological interest than the target compound itself (This point will be discussed in more detail in the animal distribution studies section.).

It was later found that the 2-chloroethylglucosyl urea (XLIII) could be successfully prepared in a 76% yield by a heterogeneous reaction between an aqueous solution of D-2-glucosamine and a solution of 2-chloroethyl isocyanate in chloroform at 0°C. That no carbamate formation had occurred was evident in the absence of any ir bands in the region of 1700-1720 cm^{-1}. Furthermore, treatment of XLIII with acetic anhydride in pyridine yielded a compound which was, in all respects, identical to XL.

Nitrosation of XLIII in aqueous ethanol with sodium nitrite/HCl gave a 51% yield of the desired streptozotocin analogue (XXXIXa). Again, the structure of this compound was confirmed by

conversion to XLI by acetylation with acetic anhydride in pyridine.

Subsequent to the synthesis of XLI in this study, Schein and co-workers from the National Cancer Institute reported that this compound possesses significant antitumor activity.[63] No comment was made by these authors as to their source of XLI other than that it was "newly synthesized." Furthermore, the procedure for synthesis and physical properties of this compound have not yet appeared in the literature.

Glucose Tolerance Test Results

Since *in vivo* deacetylations of acetylated drugs is a common occurrence,[64] (in fact, the antileukemic activities of streptozotocin and its tetra-acetylated analogue are approximately equal[65]) it was decided to submit compounds XL and XLI to the glucose tolerance test. The results of this test are shown in Figure V. Both XL and XLI displayed marked hyperglycemic activity, and, in fact, at 60 minutes XL possesses biological potency equal to that of streptozotocin itself. However, neither of the compounds caused permanent diabetes at the dose level studied. It was somewhat surprising that the non-nitrosated compound (XL) showed any activity at all. Quite possibly the β-cell toxicity is caused by the liberated diazomethane while some secondary hyperglycemic effect (and thus the pancreatotrophic character) can be attributed to the glucosylurea structure itself.

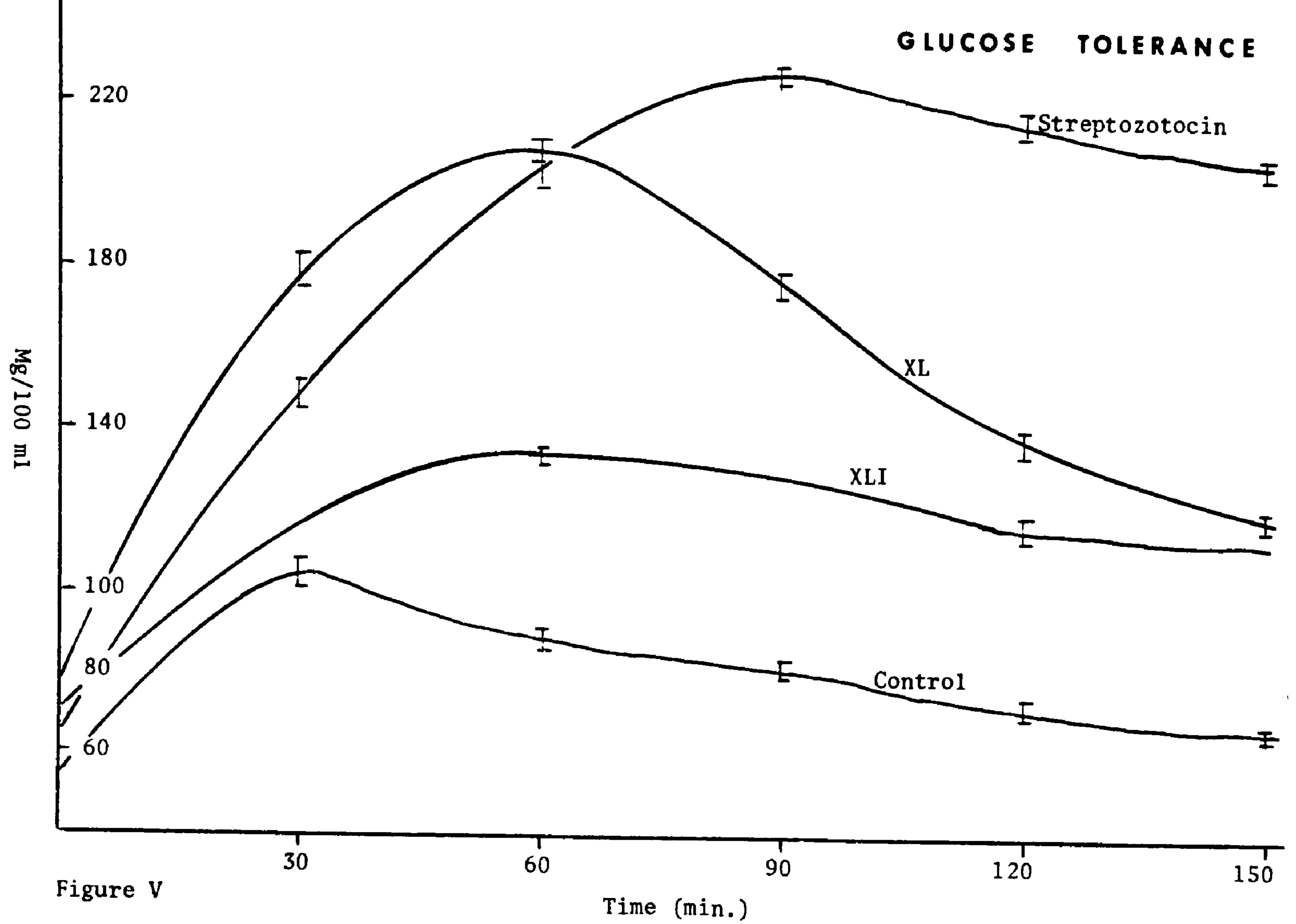

Figure V

Aryl Streptozotocin Analogues

A second procedure for the preparation of ^{131}I labeled radiopharmaceuticals is the electrophilic iodination of activated aromatic compounds with ^{131}I-iodine monochloride. Since no aryl streptozotocin analogues have as yet been evaluated for diabetogenic activity, a number of compounds of this type have been prepared (these are listed below) and submitted to McNeil Laboratories for screening as hyperglycemics.

#	R	mp	% yield
XLIII	4-OCH_3	192-194°C	91
XLIV	3-OCH_3	202-203°C	87
XLV	2-OCH_3	201.5-202°C	83
XLVI	2-OCH_2CH_3	202-203°C	87
XVII	4-CH_3	196-197°C	89
XLVIII	H	203.5-204°C	91
XLIX	4-NO_2	222°C dec	94
L	3-NO_2	198°C	88
LI	4-NH_2	208.5°C	95
LII	3-NH_2	200.5°C	87

In an attempt to devise a synthesis of these compounds which would involve fewer steps than required to prepare the 2-chloroethyl analogue (XL, Scheme VII), p-methoxyphenyl isocyanate was reacted with a solution of glucosamine in pyridine. This solution was then treated with acetic anhydride to give a 31.5% yield of the imidazolidone (LIII). Herr and co-workers reported the

LIII

LIV

formation of the structurally similar compound (LIV) when streptozotocin was treated with aqueous sulfamic acid.[54] Since this procedure did not yield the desired product, compounds XLIII through L were prepared by the same basic procedure as that outlined in Scheme VII.

In an attempt to prepare the N-nitroso compound LV, the urea XLIII was treated with sodium nitrite/HCl in acetic acid.

LV

LVI

Table XII[a]

^{131}I-XLIII tissue distribution

1. Injected in H_2O

	15 minutes[†]	30 minutes[†]	60 minutes[†]	3 hours[††]	5 hours[††]
Lung	0.761 ± 0.119	0.514 ± 0.053	0.322 ± 0.060	0.174 ± 0.009	0.125 ± 0.027
Liver	1.231 ± 0.133	0.764 ± 0.086	0.429 ± 0.046	0.120 ± 0.019	0.081 ± 0.014
Spleen	0.621 ± 0.088	0.541 ± 0.082	0.371 ± 0.038	0.152 ± 0.011	0.091 ± 0.016
Kidney	4.378 ± 0.636	2.901 ± 1.105	1.448 ± 1.074	0.209 ± 0.011	0.127 ± 0.030
Pancreas	0.517 ± 0.050	0.347 ± 0.031	0.207 ± 0.019	0.107 ± 0.001	0.068 ± 0.017
Blood	0.772 ± 0.331	0.573 ± 0.139	0.309 ± 0.078	0.226 ± 0.031	0.173 ± 0.041
Pancreas/Liver	0.420	0.454	0.483	0.892	0.840

2. Injected in DMSO

	15 minutes	30 minutes[†]	60 minutes[†]	3 hours[†]
Lung	0.851 ± 0.045	0.711 ± 0.071	0.642 ± 0.112	0.348 ± 0.049
Liver	0.484 ± 0.033	0.416 ± 0.031	0.357 ± 0.082	0.220 ± 0.041
Spleen	0.650 ± 0.083	0.561 ± 0.067	0.493 ± 0.077	0.237 ± 0.021
Kidney	0.802 ± 0.091	0.671 ± 0.108	0.622 ± 0.097	0.349 ± 0.056
Pancreas	0.499 ± 0.037	0.408 ± 0.073	0.320 ± 0.071	0.169 ± 0.039
Blood	1.057 ± 0.078	0.856 ± 0.083	0.802 ± 0.134	0.477 ± 0.066
Pancreas/Liver	1.031	0.983	0.898	0.768

† Six rats
†† Two rats
a See Reference 36
* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

Table XIII[a]

^{131}I-XXXIXa tissue distribution

	15 minutes	30 minutes	1 hour	3 hours	5 hours
Lung	0.902 ± 0.092	0.817 ± 0.123	0.682 ± 0.056	0.407 ± 0.043	0.297 ± 0.011
Liver	0.512 ± 0.008	0.487 ± 0.069	0.361 ± 0.044	0.256 ± 0.015	0.178 ± 0.003
Spleen	0.601 ± 0.014	0.569 ± 0.046	0.472 ± 0.033	0.270 ± 0.025	0.189 ± 0.012
Kidney	0.815 ± 0.050	0.724 ± 0.106	0.664 ± 0.042	0.421 ± 0.027	0.297 ± 0.018
Pancreas	0.404 ± 0.028	0.354 ± 0.047	0.299 ± 0.032	0.201 ± 0.025	0.119 ± 0.011
Blood	1.234 ± 0.076	1.082 ± 0.165	0.849 ± 0.068	0.587 ± 0.042	0.407 ± 0.030
Pancreas/Liver	0.789	0.727	0.828	0.785	0.669

Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

a See Reference 36.

Table XIV[a]

^{131}I-XL tissue distribution

1. Injected in propylene glycol

	30 minutes	60 minutes	3 hours	4 hours[ƒ]	5 hours[ƒ]
Lung	1.064 ± 0.092	0.782 ± 0.046	0.441 ± 0.070	0.361 ± 0.016	0.291 ± 0.050
Liver	0.926 ± 0.127	0.583 ± 0.039	0.253 ± 0.037	0.208 ± 0.066	0.185 ± 0.056
Spleen	0.724 ± 0.090	0.565 ± 0.028	0.275 ± 0.075	0.278 ± 0.028	0.214 ± 0.046
Kidney	1.886 ± 0.163	1.212 ± 0.094	0.422 ± 0.065	0.365 ± 0.016	0.312 ± 0.106
Pancreas	0.657 ± 0.039	0.524 ± 0.025	0.250 ± 0.020	0.222 ± 0.006	0.174 ± 0.039
Blood	1.083 ± 0.047	0.830 ± 0.076	0.563 ± 0.070	0.493 ± 0.016	0.400 ± 0.085
Pancreas/Liver	0.710	0.891	0.988	1.067	0.941

ƒ Two rats

a See Reference 36

2. Injected in 40% EtOH

	30 minutes	60 minutes	3 hours
Lung	1.014 ± 0.059	0.804 ± 0.098	0.458 ± 0.019
Liver	1.057 ± 0.069	0.718 ± 0.057	0.290 ± 0.022
Spleen	0.742 ± 0.146	0.655 ± 0.025	0.344 ± 0.022
Kidney	2.585 ± 0.415	1.779 ± 0.532	0.448 ± 0.041
Pancreas	0.474 ± 0.221	0.469 ± 0.136	0.236 ± 0.049
Blood	1.123 ± 0.039	0.873 ± 0.095	0.584 ± 0.037
Pancreas/Liver	0.448	0.653	0.814

Table XIV (Continued)

3. Injected in DMSO

	30 minutes ǂ	60 minutes ǂǂ	3 hours ǂǂǂ
Lung	1.048 ± 0.238	0.933 ± 0.138	0.681 ± 0.247
Liver	0.447 ± 0.048	0.366 ± 0.046	0.310 ± 0.082
Spleen	0.609 ± 0.083	0.503 ± 0.060	0.351 ± 0.094
Kidney	0.842 ± 0.120	0.691 ± 0.117	0.518 ± 0.147
Pancreas	0.450 ± 0.107	0.434 ± 0.200	0.203 ± 0.084
Blood	1.050 ± 0.161	0.879 ± 0.107	0.681 ± 0.210
Pancreas/Liver	1.007	1.186	0.655

ǂ Seven rats

ǂǂ Eight rats

ǂǂǂ Six rats

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

Table XV[a]

^{131}I-XLI tissue distribution

Injected in DMSO

	15 minutes⨍	30 minutes	60 minutes	3 hours	5 hours⨍
Lung	1.212 ± 0.129	0.916 ± 0.054	0.704 ± 0.025	0.342 ± 0.086	0.310 ± 0.012
Liver	0.622 ± 0.061	0.503 ± 0.054	0.378 ± 0.033	0.189 ± 0.032	0.183 ± 0.018
Spleen	0.705 ± 0.032	0.545 ± 0.058	0.436 ± 0.023	0.199 ± 0.043	0.196 ± 0.008
Kidney	1.020 ± 0.088	0.826 ± 0.127	0.562 ± 0.047	0.278 ± 0.015	0.285 ± 0.081
Pancreas	0.492 ± 0.103	0.437 ± 0.065	0.327 ± 0.056	0.145 ± 0.029	0.151 ± 0.040
Blood	1.406 ± 0.062	0.872 ± 0.223	0.672 ± 0.152	0.335 ± 0.034	0.381 ± 0.090
Pancreas/Liver	0.791	0.869	0.865	0.767	0.825

⨍ Two rats

a See Reference 36

* Data expressed as % dose/gm of tissue at times indicated. Except where indicated to the contrary, the value expressed is the mean of 4 rats. The number behind the ± sign represents the standard deviation. The values were normalized to standard 200g rats.

The product actually obtained from this reaction (in a 63% yield) was shown by its ir and nmr spectra and elemental analyses to be the ring nitrosated compound (LVI). The amino compounds (LI and LII) were prepared in 95 and 87% yields respectively by catalytic hydrogenation of the corresponding nitro compounds.

The aryl streptozotocin analogues chosen to be synthesized were mainly those which would be expected to undergo electrophilic iodination with iodine monochloride. The nitro compounds (XLIX and L) would not be expected to react with iodine monochloride under mild enough conditions to avoid decomposition of the sugar portion of the molecule. However, these compounds were required for the synthesis of the amino analogues (LI and LIII which should undergo electrophilic iodination under very mild conditions. Furthermore, the range of aryl substituents prepared should allow for an approximation of the structure activity relationships when glucose tolerance test results are available.

The final streptozotocin analogue prepared (LVII) combines both the glucosylurea portion characteristic of streptozotocin itself and the tosylurea portion characteristic of tolbutamide. The combination of tetra-O-acetylglucosamine and

LVII

p-tolueneisocyanate in chloroform gave a nearly quantitative yield of the product. Rat glucose tolerance measurements will confirm or refute a pancreatotrophic activity of the material. Furthermore, although the deactivated aryl ring may resist labeling with electrophilic radioiodine, a "tagged" material for radiopharmaceutical assay could be obtained by synthesis with an emitting sulfur isotope or with a chloromethylbenzenesulfonylisocyanate whose halogen could later be replaced by ^{131}I. Animal studies with this compound are underway.

IR and NMR Spectra of Streptozotocin Analogues

The ir spectra of these streptozotocin analogues has proven useful in monitoring the progress of the nitrosation reactions. In the alkyl substituted, non-nitrosated compounds (XLII

	R	R^1	R^2
XLIII	H	H	$-CH_2CH_2Cl$
XL	Ac	H	$-CH_2CH_2Cl$
XXXIXa	H	N=O	$-CH_2CH_2Cl$
XLI	Ac	N=O	$-CH_2CH_2Cl$
XLVI	Ac	H	2-ethoxyphenyl
XLIII	Ac	H	4-methoxyphenyl

and XL) the urea carbonyl is evident at 1630 cm^{-1}. Nitrosation on the urea nitrogen induces a shift toward higher energy of approximately 70 cm^{-1} for the urea carbonyl absorption (1710 cm^{-1} for XLI and 1690 cm^{-1} for XXXIXa). Therefore, the completeness of the reaction can be determined by the disappearance of the peak at 1630 cm^{-1}. In the aryl analogues, the urea carbonyl generally appeared at 1660 ± 20 cm^{-1}. When an attempt was made to nitrosate the 4-methoxyphenyl analogue (XLIII, urea carbonyl at 1665 cm^{-1}), it was expected that the urea carbonyl of the product would be shifted toward higher energy (e.g. to at least 1700 cm^{-1}). The product obtained from this reaction, in fact, showed a shift of this peak to 1640 cm^{-1}, suggesting that nitrosation had occurred on the aromatic moiety. That the substitution had, indeed, occurred on the benzene ring was confirmed by the nmr spectrum which showed the presence of only 3 aromatic protons on integration.

An interesting artifact was noted in the ir spectrum of the 4-methoxyphenyl analogue (XLIII). When this compound was first prepared, it was purified by recrystallization from a mixture of chloroform and ether. The urea carbonyl of this product appeared at 1635 cm^{-1}. Recrystallization from aqueous ethanol yielded a compound which showed a urea carbonyl absorption at 1665 cm^{-1}. The nmr spectra for both compounds were identical as were their solution ir spectra in chloroform. Furthermore, recrystallization from chloroform of the compound obtained from aqueous ethanol again yielded a product with a urea carbonyl at 1635 cm^{-1}. It would seem that this compound crystallizes in

two different crystalline modifications from these two solvent systems.

The nmr spectrum of the 2-ethoxyphenyl analogue (XLVI) is shown in Figure VI. The nmr spectrum of pentaacetyl-β-D-glucose[65] was used as a model for the interpretation of the nmr of XLVI. The ethoxy group appears as a 3 proton triplet (H_a, $\delta = 1.30$) and a 2 proton quartet (H_e $\delta = 4.05$) partially obscured by ring proton signals (H_c and H_d) which integrate for 2 protons. The four acetyl methyls appear as a group of singlets (H_b, $\delta = 2.0$) integrating for 12 protons. The multiplet (centered at $\delta = 5.17$) is the result of two overlapping quartets with equal coupling constants of 9 cps, corresponding to H_g and H_i. The aromatic protons are evident as two multiplets, the first (H_j, $\delta = 6.87$) integrating for 3 protons and the second (H_i, $\delta = 8.28$) integrating for 1 proton.

The anomeric proton appears as a doublet (H_j, $\delta = 5.84$) integrating for 1 proton and having a coupling constant of 9 Hz. The fact that this doublet integrates for a full proton and has a J value of 9 Hz is taken to mean that this compound was obtained as the pure β-anomer. The anomeric proton for pentaacetyl-β-D-glucose appears as a doublet with a coupling constant of 8 Hz and that of the α-anomer has a coupling constant of 3 Hz.[65] Furthermore, it has been reported that for six membered cyclic compounds the coupling constants for vicinal axial-axial protons may range from 6 to 14 Hz while the coupling constants for

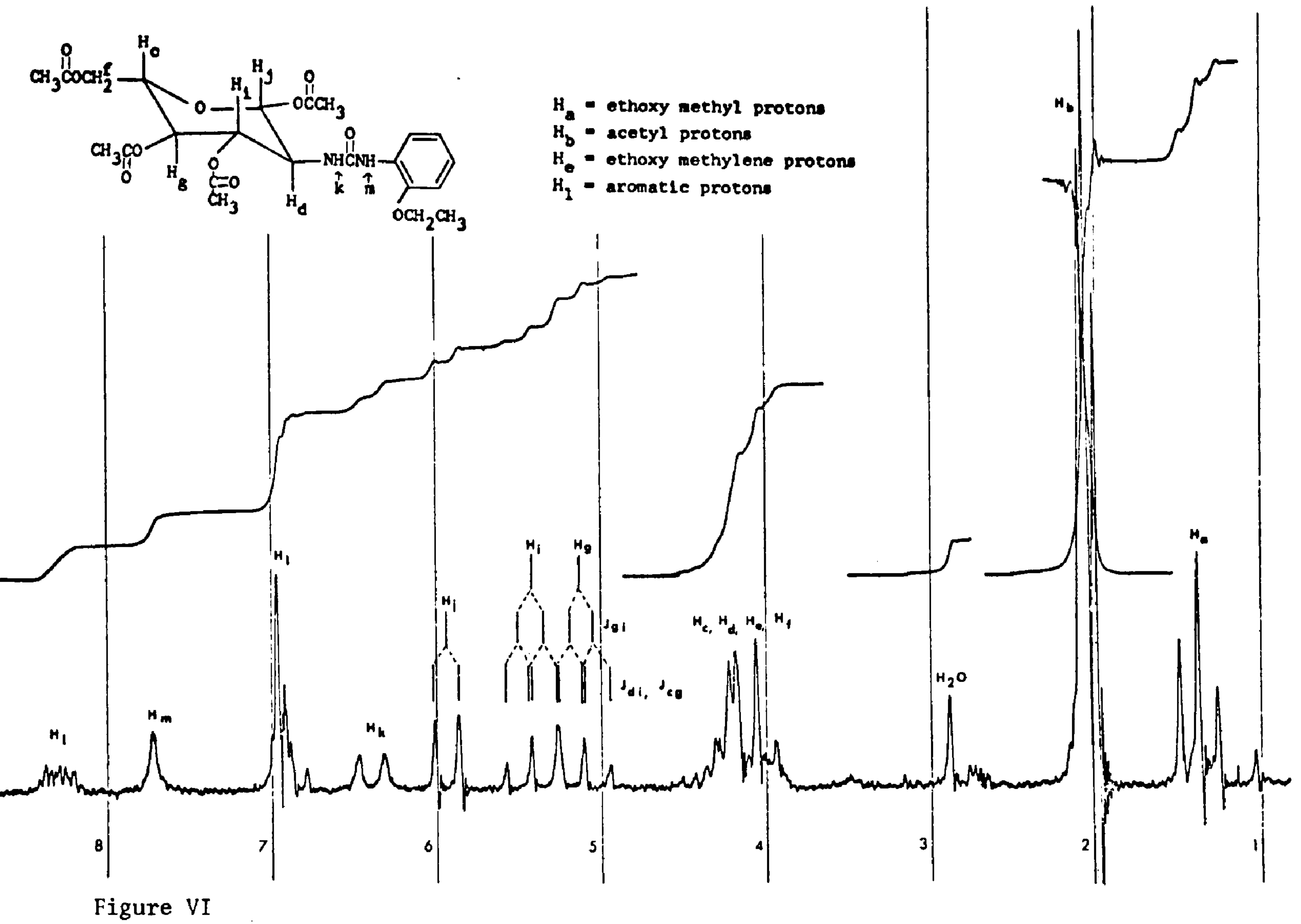

Figure VI

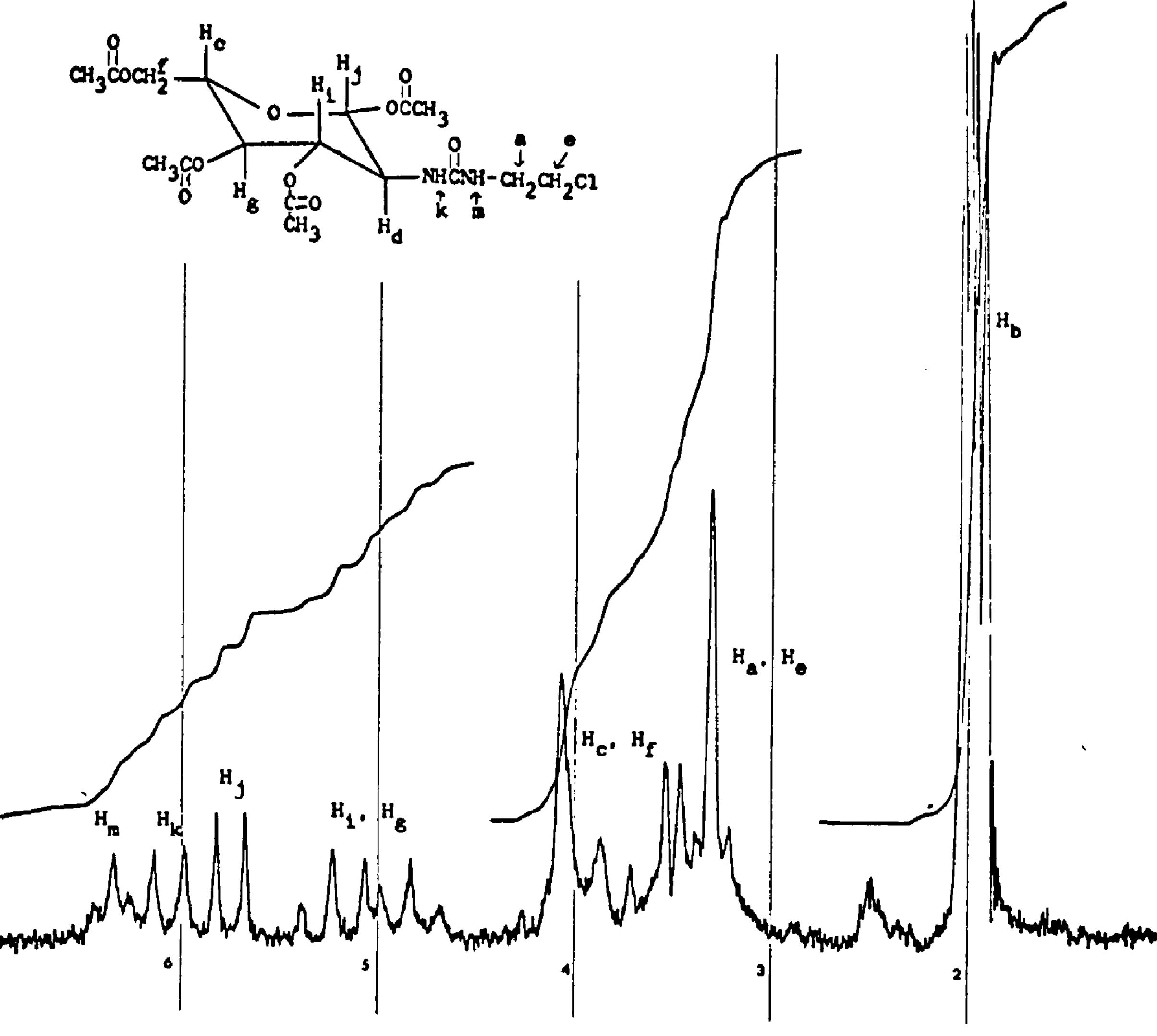

Figure VII

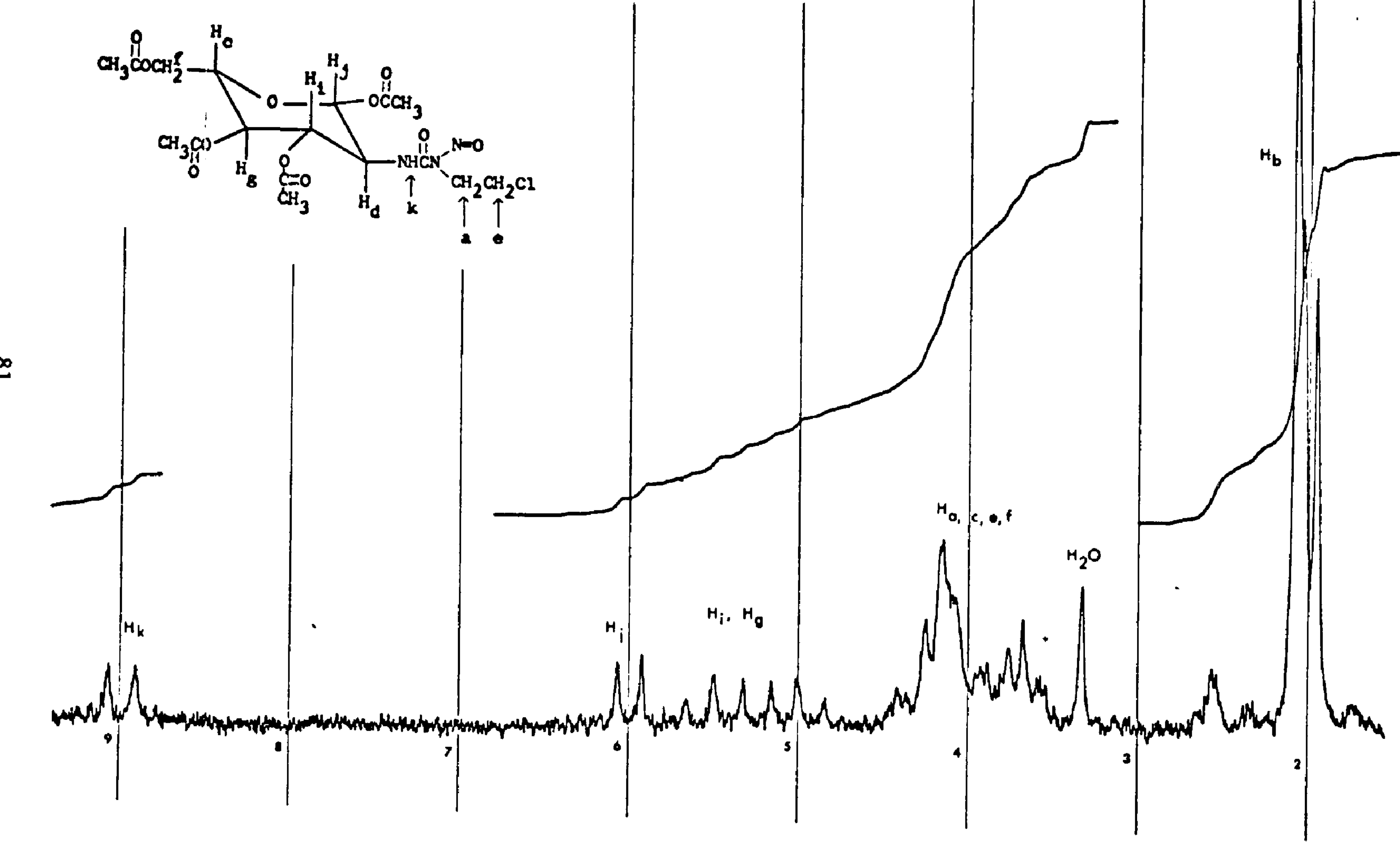

Figure VIII

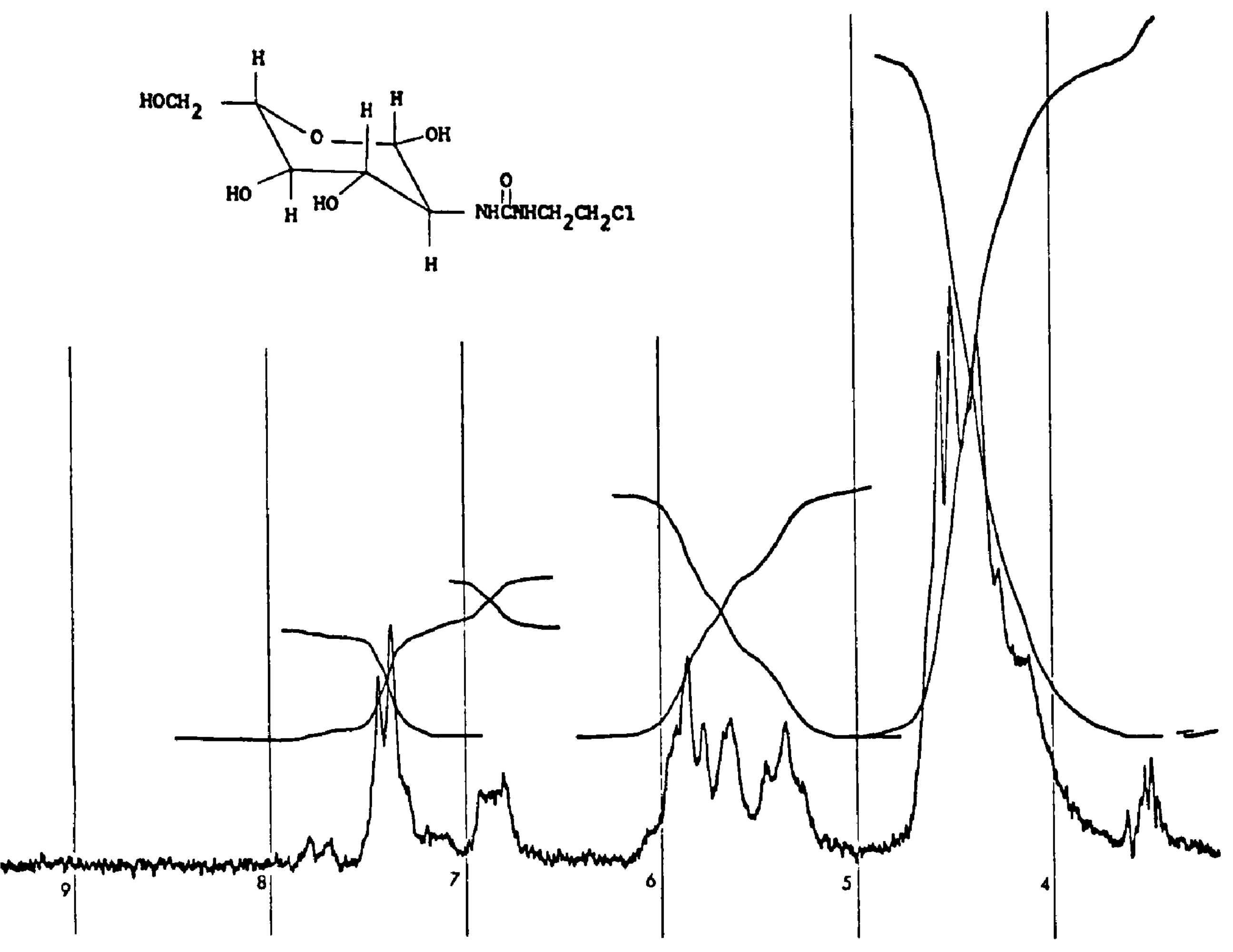

Figure IX

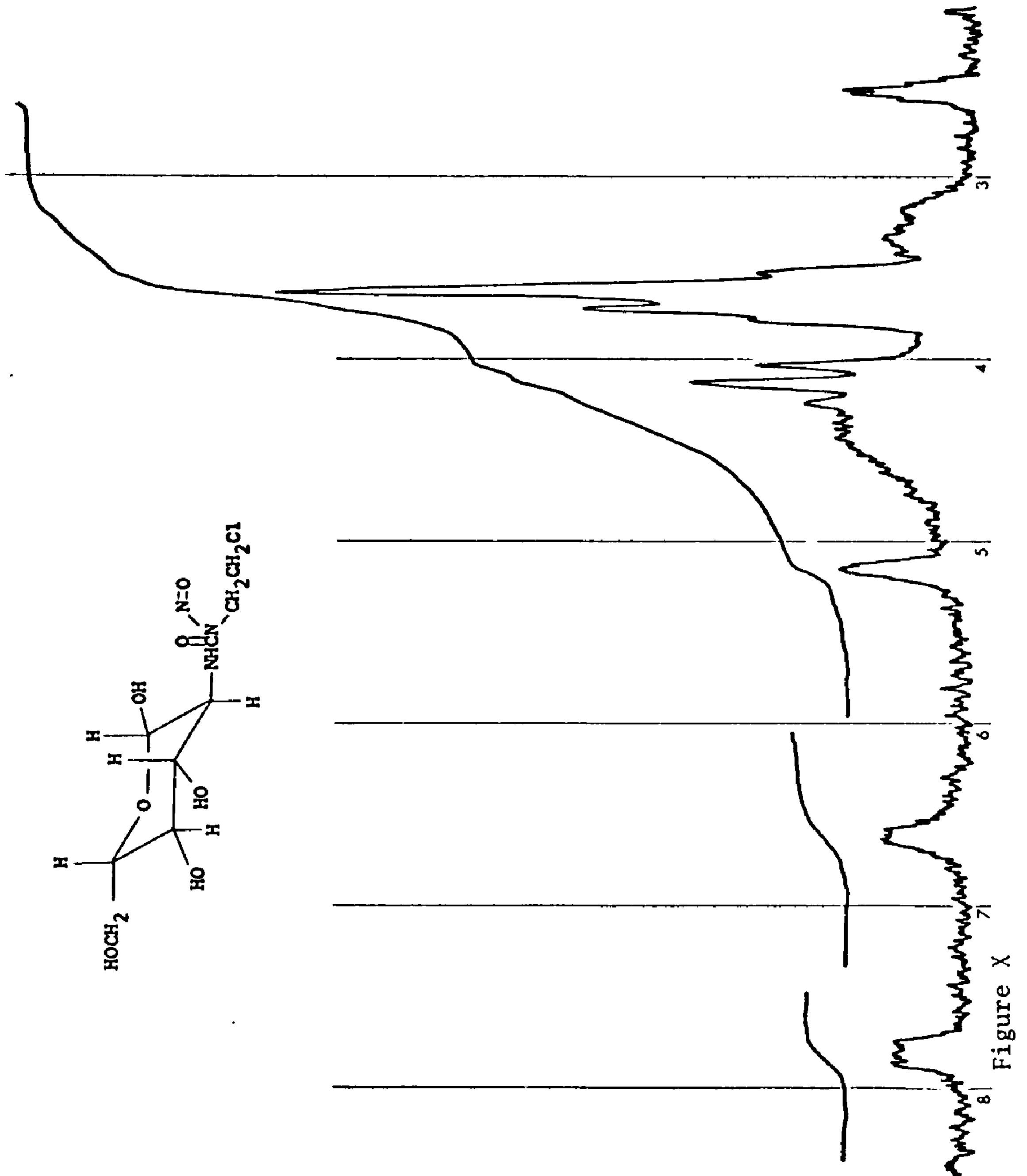

Figure X

vicinal axial-equatorial protons may vary from 0 to 5 Hz. Furthermore, since the coupling constant for the anomeric proton in all of the streptozotocin analogues prepared was 9 Hz, it would seem that all of these compounds are pure β-anomers.

In their original synthesis of tetra-O-acetyl-D-glucosamine, Bergmann and Zervas indicated that they believed that their compound was obtained as the β-anomer. They based this conclusion on the fact that upon acetylation of the tetracetyl material they obtained a product which was identical (mp and specific rotation) with a compound previously reported as β-pentaacetyl-D-glucosamine by Hudson and Dale.[66] The two anomers of pentaacetyl-D-glucosamine were first prepared in 1899 by Lobry de Bruyn and Van Eckenstein.[67]

Hudson and Dale[66] based their assignment on a similarity in specific rotation of their penatacetyl-D-glucosamine with that of β-pentaacetyl-D-glucose. In 1955, Micheel and co-workers[68] reported the synthesis of the α-anomer of tetra-O-acetyl-D-glucosamine by the treatment of β-pentaacetyl-D-glucosamine with HBr in acetic acid. They reported, as further evidence for the correctness of their assignment of the α-structure to their product, the fact that the compound they had obtained yielded an oxazoline (LVIII) when heated in methanol. They further stated that only the α-form of tetra-O-acetyl-D-glucosamine could undergo such a cyclization. Although the assignment of their product as the α-anomer appears to be correct, on the basis of nmr data

collected herein, it would seem from consideration of cyclohexane stereochemistry and molecular models that both anomers could yield an oxazoline. Thus, Micheel's aasignment of anomeric structure was fortuitously correct and can now be supported by analysis of the coupling constants for the protons on C#1 and C#2 as discussed herein. It is significant that only the nmr spectra of the acetylated streptozotocin analogs are interpetable. The acetyl moieties shift the carbon-bound protons of the glucose ring sufficiently to permit individual analyses (see nmr spectra, Figure VII). The de-acetylated sugars (see nmr spectra, Figure IX) display such considerable overlap of the C-H resonances that, at least at 60 megaHz, a discrete interpretation is not possible.

Not only does analysis of the nmr spectra provide evidence as to the anomeric form of the streptozotocin derivatives that have been prepared, but it also establishes the site of nitrosation. Two non-equivalent urea nitrogens might serve as the site of NO incorporation.

The nmr spectra of compound XL (Figure VII) and compound (XLI) clearly indicated that nitrosation had occurred on the desired nitrogen (i.e. on the nitrogen attached directly to the chloroethyl side chain). The urea NH_K again is seen as a doublet at $\delta = 6.10$, being coupled to the glucosyl proton, H_d. The remaining urea proton (H_m) being coupled to 2 protons (H_a) appears as a triplet at $\delta = 6.38$ (see Figure VII). After nitrosation, the triplet (H_m) disappears, while the doublet (H_k) is shifted

downfield to δ = 8.99, thus indicating that nitrosation had occurred on the nitrogen atom indicated. Since acetylation of compound XXXIXa yields XLI, this establishes that in the synthesis of XXXIXa, nitrosation has also occurred on the nitrogen attached directly to the chloroethyl side chain.

Labeling of Streptozotocin Analogues

In an attempt to prepare the iodo compound (LIX), the corresponding chloro compound (XL) was refluxed with an excess

CH_2OAc ... CH_3

LVIII

CH_2OAc ... $NHCNH-CH_2CH_2-I$

LIX

of sodium iodide in anhydrous acetone for 24 hours. It was anticipated that the replacement of the chlorine atom by iodine would result in a detectable shift of the adjacent methylene protons in the nmr spectrum, thus allowing for the determination of the percent of halogen exchange which had occurred. However, the nmr spectrum of the product obtained was virtually identical to the nmr spectrum of the starting material. This result was not totally unexpected, since it has been reported that the shift accompanying such a change in substituents (Cl→I) can be as little as 0.03 ppm or as much as 1.05 ppm.[30] The ir spectrum of the product of this reaction was also identical with that of the starting

material; however, the combustion analysis indicated that approximately 25% of the chlorine had been replaced by iodine. The fact that no change was noted in the ir or nmr spectra is interpreted to mean that the remainder of the molecule had remained intact during the reaction. This same procedure was also employed to prepare the ^{131}I labeled analogue of LVIII for use in animal distribution studies -- in this case, refluxing was only continued for 3 hours. Such short contact times would certainly not decompose the sugar moiety since in the iodide exchange with $Na^{127}I$ the molecule survived 24 hours of reflux.

Similarly, when the nitrosated compound (XLI) was iodinated with non-radioactive NaI under the identical conditions employed for the non-nitrosated compound (XL), there was no change in its nmr or ir spectra. Thus it appears that in these chloroethyl systems, conversion to an iodoethyl counterpart effects negligible spectral changes. When radioiodide ($Na^{131}I$) was employed with the nitrosated (XLI) it was apparent that exchange had taken place for a "hot" organic product was isolated.

An attempt to label the corresponding non-acetylated compounds (XLIII and XXXIXa) by this procedure resulted in apparent decomposition of both substances. Furthermore, when animal tissue-distribution studies were preformed on the oily "products" which resulted from these labeling reactions they gave totally unreproducible results. It was later found that these compounds could, indeed, be labeled without decomposition by stirring in DMSO with ^{131}I-sodium iodide at room temperature.

Several attempts were made to iodinate the 4-methoxyphenyl analogue (XLIII) with iodine monochloride. When this reaction was performed at room temperature in acetic acid, only recovered starting material was isolated. An attempt to accelerate the reaction by heating to 40°C resulted in decomposition of the starting material. The above reaction was repeated in THF at room temperature containing a catalytic amount of boron trifluoride etherate. This too resulted in decompositon of starting material.

When the 3-methoxyphenyl analogue (XLIV) was iodinated in acetic acid at room temperature with iodine monochloride, a compound was obtained which was shown by nmr to be partially substituted with iodine on the aromatic nucleus. The iodine uptake was incomplete.

Tissue Distribution Studies

Tissue-distribution studies were performed on the 2-chloroethyl streptozotocin analogues (XLII, XL, XXXIXa and XLI). All of these compounds were labeled with ^{131}I by nucleophilic displacement of the chlorine atom by ^{131}I-sodium iodide (XL and XLI were labeled in refluxing anhydrous acetone, while XLIII and XXXIXa were labeled in DMSO at room temperature). Each of the labeled compounds was purified by passage through an anion exchange column to remove ionic ^{131}I.

Table XII-1 shows the results obtained when an attempt was made to label XLIII in refluxing acetone. The standard deviations observed in this case were much higher than those normally obtained. These high errors are believed to be due to

decomposition of the glucose portion of the molecule as a result of the more vigorous labeling conditions used in this labeling procedure. When this same compound was labeled in DMSO at room temperature, considerably more reproducible results were obtained (see Table XII-2). A rapid peaking of pancreatic uptake and of the pancreas/liver ratio was observed. This result was in accord with the findings reported by Beierwaltes,[69] who found, using ^{14}C-streptozotocin that the pancreatic uptake "peaked" at approximately 5 minutes. Unfortunately, there was poor partitioning of this agent (^{131}I-XLIII) from the blood, and blood levels remained more than two times that of pancreas levels throughout the time of the study.

Table XIII shows the tissue distribution results for ^{131}I-XXXIXa. In this case, the pancreas/liver ratio was maximum after approximately 1 hour at a value of 0.828. Again, the activity in the blood remained considerably higher than that in the pancreas. The low pancreas/liver ratio and the high blood activity makes this agent of little practical use as a visualizing agent for normal pancreatic tissue.

Since compound ^{131}I-XL was insoluble in water, it was necessary to employ a different solvent for injection. This substance was found to be soluble in both propylene glycol and DMSO and slightly soluble in 40% aqueous ethanol. All three of these solvents can be used as injection solvents in animals, but DMSO has not yet been approved for human use. Propylene glycol was found to be the most suitable injection medium, even

though this solvent promoted a substantially higher liver activity than DMSO at shorter time periods. DMSO was found to be an unsuitable injection solvent for this particular compound even though this solvent gave the highest pancreas/liver ratios. This compound (^{131}I-XL), while innocuous to the animals when injected in propylene glycol or 40% ethanol, was found to be acutely toxic in DMSO. The reason for this toxicity is not known, although convulsions were occasionally noticed in the animals immediately after injection, suggestion some action on the central nervous system (CNS). DMSO has been reported to enhance drug action on the CNS.[70] One possible explanation for this CNS activity might be a hypothetical cyclization of XL to form the imidazolidone type compound (LX). This imidazolidone analogue, if formed in situ, would be closely related to known CNS-active hydantoins

LX

and oxazolidones.[71]

As previously mentioned, such a cyclization did not occur during labeling with sodium iodide in refluxing acetone since the nmr spectrum of the product of this exchange reaction still displayed the presence of all four acetyl groups. However,

the possibility of such a ring closure in DMSO cannot be ruled out at this point.

After labeling with ^{131}I, compound XLI was found to have sufficient solubility in only one solvent, DMSO. Therefore, this solvent was used as an injection medium. The results of the distribution studies for this compound are shown in Table XV. This distribution closely parallels that of compound XXXIXa (Table XIV-3) with the exception of an elevated liver uptake which results in a lower pancreas/liver ratio.

Even though the results of the organ distribution studies have not yielded the desired pancreas/liver ratio of 3:1, the value of approximately 1:1 is viewed as promising. Since compound XLI has also been shown to possess significant antitumor activity, it might be expected that even higher target-background ratios will be found when these agents are tested in tumor-bearing animals, especially those bearing pancreatic tumors.

In preliminary tissue distribution studies of these compounds in Golden Syrian hamsters with β-cell adenocarcinoma of the pancreas,[72] pancreas tumor to liver ratios of approximately 3 have been obtained. This testing data has not yet been completed and, as a result, is not included in this report.

EXPERIMENTAL SECTION

Equipment and Analyses

Melting points were determined on a MelTemp or a Thomas Hoover melting point apparatus and are reported uncorrected. Combustion analyses were performed by Dr. G. I. Robertson of Florham Park, New Jersey. The infrared spectra were obtained on either a Perkin-Elmer 257 spectrophotometer or a Beckman IR-33. The samples were run in the neat state, or as Nujol mulls or in solution using 0.1 mm sodium chloride liquid cells compensated with pure solvent. The nmr spectra were recorded on a Hitachi Perkin-Elmer R-20A spectrometer using tetramethylsilane as the internal standard.

The animal tissue distribution studies were performed at Hahnemann Medical School's Department of Nuclear Medicine, with the assistance of Ms. Marlynne Micalizzi. A number of these studies were performed by Mr. Victor Risch. The radioactive tissue samples and chromatographic samples were counted in a Baird Atomic Atomatic Well Counter.

The glucose tolerance tests were performed by Dr. G. F. Tutwiler of the Pharmacology Laboratory of McNeil Laboratories, Fort Washington, Pa.

Part I: Synthesis of Candidate Radiopharmaceuticals

Carbutamide (VIIa)

Method A. This compound was prepared from sulfanilamide and methyl n-butylcarbamate as described by Lautenberg and Zurini.[28] The product was obtained in a 65% yield; mp 135-137°C (literature[28] 139-142°C).

Method B. Carbutamide was also prepared from sulfanilamide and n-butyl isocyanate as described by Fujii and Junichiro.[27] This procedure resulted in formation of the product in an 87% yield; mp 138-139°C (literature[27] 138-140°C).

Reaction of Carbutamide with Cyanoguanidine

A suspension containing carbutamide (1.27 g, 4.68 mmol), cyanoguanidine (0.40 g, 4.76 mmol) and pyridine hydrochloride (0.72 g, 6.23 mmol) in 4 ml of pyridine was protected from atmospheric moisture with a $CaCl_2$ drying tube and refluxed for 4 hrs. The resulting homogeneous solution was cooled to room temperature and poured into 100 ml of Et_2O. The oil which separated was collected and triturated first with Et_2O and then with pentane but crystallization did not ensue. Upon trituration with H_2O, white crystals formed which were collected and washed with anhydrous EtOH. These crystals were shown by ir and nmr to be sulfanilamide and were obtained in a 61% (0.49 g) yield.

Reaction of Carbutamide Hydrochloride with Cyanoguanidine

A solution of carbutamide hydrochloride (1.43 g, 4.64 mmol) and cyanoguanidine (0.4 g, 4.75 mmol) in 3 ml of anhydrous pyridine was protected from atmospheric moisture by a $CaCl_2$ drying tube and refluxed for 3 hr. Upon cooling to room temperature, the solution deposited 0.26 g of white crystals which were shown by their ir spectrum to be cyanoguanidine (65%). The filtrate was triturated with CH_2Cl_2 resulting in the formation of 0.86 g of white crystals. These crystals were demonstrated by their ir and nmr spectra to be carbutamide. Isolation of 0.86 g of carbutamide accounts for 68% of the carbutamide hydrochloride utilized.

1(4-Amino-3-iodobenzenesulfonyl)-3-butyl Urea Hydrochloride (XI)

Iodine monochloride (1.25 g, 7.69 mmol) in 10 ml of HOAc was added dropwise to a solution of carbutamide (2.0 g, 7.37 mmol) in 50 ml of HOAc at room temperature. After refluxing for 4 hr, the orange colored medium was cooled to room temperature and poured into 200 ml of cold water. The orange oil which separated solidified after standing for 2 days forming a brown solid. Recrystallization of this brown solid from 95% EtOH yielded 1.35 g (42.%) of white needles mp 175-176°C; ir (nujol) 3480, 3380, 3320, 3200 (NH) and 1645 cm^{-1} (urea C=O); nmr (acetone-d_6) δ1.20 (m, 7H, -$C\underline{H}_2C\underline{H}_2C\underline{H}_3$), 3.8 (m, 2H, N-$C\underline{H}_2$), 5.80 (m, 3H, N$\underline{H}$), 6.98 (d, $J_{5,6}$=9 Hz, 1H, Ar-H), 7.72 (q, $J_{5,6}$=9 Hz, $J_{6,2}$=3 Hz, N$\underline{H}$). Ar-$\underline{H}$), 8.13 (d, $J_{6,2}$=3 Hz, 1H, Ar-$\underline{H}$) and 8.21 ppm (s, 1H, N$\underline{H}$).

Anal. Calcd for $C_{11}H_{16}N_3SO_3I \cdot HCl \cdot 1/4\ H_2O$: C, 30.15; H, 4.03; N, 9.58.

Found: C, 29.92; H, 3.57; N, 9.55.

1-p-Toluenesulfonyl-3-(N,N-dimethylaminoethylaminoethyl) Urea (XIIa)

A solution of p-toluenesulfonyl isocyanate (2.0 g, 10.1 mmol) in 10 ml of dioxane was added dropwise (over a 30 min period) to N,N-dimethyldiethylenetriamine (1.33 g, 10.1 mmol) in 30 ml of dioxane at room temperature. The resulting solution was stirred at room temperature for 1 hr, during which time a white precipitate formed. The precipitate was collected by filtration, washed with ether and air dried. Recrystallization from anhydrous EtOH yielded 2.53 g (76.2%) of white hygroscopic crystals: mp 179-180°C; ir (nujol) 3490, 3390, 3270 (NH) and 1650 cm^{-1} (C=O); nmr (D_2O) δ2.30 (s, 3H, Ar-$C\underline{H}_3$), 2.52 (s, 6H, $N(C\underline{H}_3)_2$), 2.86 (m, 8H, $NC\underline{H}_3$), 2.52 (and 7.46 ppm (m, 4H, Ar-$\underline{H}$).

Anal. Calcd for $C_{14}H_{24}N_4O_3S$: C, 51.19; H, 7.36; N, 17.05.

Found: C, 51.18; H, 7.61; N, 16.82.

Thin layer chromatography on Baker-Flex silica gel 1B-F eluting with methanol-water (3:1) gave a single spot, R_f=0.29.

1-p-Toluenesulfonyl-3-(aminoethylaminoethyl) Urea (XIIb)

A solution of p-toluenesulfonyl isocyanate (3.0 g, 15.2 mmol) in 15 ml of tetrahydrofuran (THF) was added slowly -- over a 30 min period -- to freshly distilled diethylenetriamine (1.58 g, 15.6 mmol) in 70 ml of THF at room temperature. After stirring

for 30 min., the white precipitate which had formed was collected by filtration, washed with anhydrous Et_2O and dried at 65°C _in vacuo_. Recrystallization from dioxane/water yielded 3.32 g (73%) of white hygroscopic crystals; mp 135-138°C; ir (nujol) 3350, 3280 (NH), sh 1640 cm^{-1}(C=O); nmr (D_2O) δ2.24 (s, 3H, Ar-C$\underline{H}_3$), 2.88 (m, 8H, NC$\underline{H}_2$), and 7.37 ppm (m, 4H, Ar-$\underline{H}$).

Anal. Calcd for $C_{12}H_{20}N_4O_3S$: C, 47.97; H, 6.71

Found: C, 47.82; H, 6.86

Thin layer chromatography on Baker-Flex silica gel 1B-F eluting with methanol-water (19:1) gave a single spot, R_f = 0.86.

Reaction of 1-p-Toluenesulfonyl-3-(aminoethylaminoethyl) Urea with Phosgene (XIII)

A suspension of 1-p-toluenesulfonyl-3-(aminoethylamino-ethyl) urea (1.0 g, 3.33 mmol) in 25 ml of dry pyridine was treated with phosgene (.0.33 g, 3.34 mmol-2.63 g of a 12.5% phosgene in benzene solution). After heating at 60°C for 3 hr, the solution was cooled to room temperature and 25 ml of cold H_2O was added. Upon standing for 5 days at room temperature, a tan precipitate formed. Recrystallization of this precipitate from EtOH/H_2O yielded 0.57 g (52%) of tan crystals: mp 196-198°C; ir (nujol) 3390, 3360, 3080 (NH), 1700 (imidazolidone C=O) and 1660 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ2.35 (s, 3H, Ar-C$\underline{H}_3$), 3.10 (m, 8H, 4 N-C$\underline{H}_2$), 6.35 (b, 2H, N$\underline{H}$), 7.40 (m, 4H, Ar-$\underline{H}$) and 10.55 ppm (b, 1H, N$\underline{H}$).

Anal. Calcd for $C_{13}H_{18}N_4SO_4$: C, 47.84; H, 5.56

Found: C, 47.78; H, 5.54.

1-p-Toluenesulfonyl-3-carbethoxymethyl Urea (XIIc)

A solution of ethyl glycinate (3.54 g, 34.3 mmol) in 50 ml of anhydrous THF was treated dropwise with p-toluenesulfonyl isocyanate (6.3 g, 32.0 mmol in 20 ml of THF). During addition of the isocyanate, the temperature rose to 55°C. After stirring for 0.5 hr, the solution was allowed to stand at room temperature for 12 hr. Evaporation to dryness *in vacuo* left a white powder which was recrystallized from EtOH/H_2O to yield 7.2 g (75%) of white needles; mp 170-171°C; ir (nujol) 3110, 3170, 3315 (NH), 1742 (ester C=O) and 1660 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ1.15 (t, 3H, CH_3), 2.40 (s, 3H, Ar-CH_3), 3.75 (d, 2H, -CH_2), 4.05 (q, 2H, O-CH_2), 6.80 (t, 1H, NH), 7.61 ppm (m, 4H, Ar-H) and 10.70 ppm (s, 1H, NH).

Anal. Calcd for $C_{12}H_{16}N_2O_5S$: C, 47.98; H, 5.37; N, 9.32

Found: C, 48.17; H, 5.62; N, 9.17

1-p-Toluenesulfonyl-3-carboxymethyl Urea (XIIe)

A solution of 1-p-toluenesulfonyl-3-carbethoxymethyl urea (2.0 g, 6.6 mmol) in 5 ml of 10% aqueous sodium hydroxide was refluxed for 1 hr. After cooling to room temperature and acidification with concentrated HCl, a white precipitate formed which was collected by filtration and recrystallized from EtOH/H_2O. A yield of 1.7 g (88%) of white needles was obtained: mp 190°C dec; ir (nujol) 3350, 3170, 3120 (NH and OH); 1730 (acid C=O) and 1680 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 2.40 (s, 3H, Ar-CH_3), 3.86 (d, 2H, CH_2), 6.88 (t, 1H, NH), 7.62 (m, 4H, Ar-H) and 10.72 ppm (s, 1H, NH).

Anal. Calcd for $C_{10}H_{12}N_2O_5S\ H_2O$: C, 41.37; H, 4.86; N, 9.65

Found: C, 41.40; H, 4.63; N, 9.68.

1-p-Toluenesulfonyl-3-hydrazocarbomethyl Urea (XIId)

A suspension of 1-p-toluenesulfonyl-3-carboxy-methyl urea (1.0 g, 3.44 mmol) in 10 ml of thionyl chloride (16.38 g, 0.138 mol) was refluxed for 1 hr. Evaporation of the thionyl chloride *in vacuo* left a yellow solid which was added cautiously to a solution of hydrazine hydrate (2.0 ml, 4.2 mmol) in 5 ml of H_2O. After stirring for 30 min, the yellow colored medium was diluted to 30 ml and neutralized with conc HCl. The resulting white solid was collected and recrystallized from $EtOH/H_2O$ yielding 0.70 g (63%) of white crystals: mp 191-193°C; ir (nujol) 3300, 3180, 3120 (NH), 1705, 1685 (C=O) and 1650 cm^{-1} (urea C=O); nmr ($DMSO-d_6$) δ 2.32 (s, 3H, Ar-$C\underline{H}_3$), 3.52 (d, 2H, N-$C\underline{H}_2$), 6.55 (b, 6H, N$\underline{H}$) and 7.48 ppm (m, 4H, Ar-$\underline{H}$).

1-p-Toluenesulfonyl-3-(2-chloroethyl) Urea (XIV)

A solution of p-toluenesulfonamide (4.29 g, 25.04 mmol) in 6 ml of 5 N NaOH (30 mm) was treated with a solution of 2-chloroethyl isocyanate in 100 ml of 1:1 $CHCl_3:Et_2O$ at 0°C. The above mixture was stirred vigorously at 0°C for 4.5 hr. After gradual warming to room temperature, 20 ml of H_2O was added and the two phases separated. The aqueous phase was acidified with conc HCl and the precipitate which formed was collected and recrystallized,$EtOH/H_2O$. A yield of 3.19 g (46%) of white needles

was obtained: mp 150-152°C; ir (nujol) 3340, 3110 (NH) and 1665 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 2.38 (s, 3H, Ar-C$\underline{H}_3$), 3.45 (m, 4H, N-C$\underline{H}_2$C$\underline{H}_2$Cl), 6.71 (t, 1H, N$\underline{H}$), 7.42 (d, 2H, Ar-$\underline{H}$), 7.79 (d, 2H, Ar-$\underline{H}$) and 10.70 ppm (s, 1H, N$\underline{H}$).

Anal. Calcd for $C_{10}H_{13}N_2SO_3Cl$: C, 43.40; H, 4.73; N, 10.12.

Found: C, 43.54; H, 4.76; N, 10.04.

2-Benzenesulfonamidopyrimidine

This compound was prepared from benzenesulfonyl chloride and 2-aminopyrimidine by the procedure of English *et al.*[73] The product was obtained in an 89.5% yield. Recrystallization from 7:3 EtOH:H_2O gave white needles: mp 228-230°C (literature 229-230°C).

Attempted Nitration of 2-Benzenesulfonamidopyrimidine

Method A. To a solution of 1.5 ml of fuming nitric acid in 2.2 ml of conc H_2SO_4 was added cautiously 2-benzenesulfonamidopyrimidine (1.0 g, 4.25 mmol). The resulting yellow medium was heated at 60°C for 3 hr. After cooling the contents of the flask to room temperature, the solution was poured into 200 ml of cold H_2O, resulting in the formation of a yellow precipitate which was collected and washed with anhydrous EtOH and Et_2O. The nmr spectrum of this compound indicated that the product obtained was 2-(m-nitrobenzenesulfonamido)pyrimidine:

mp 215-218°C (Literature[32] 217-218°C).

Method B: Three ml of conc H_2SO_4 was cooled to 0°C and 2-benzenesulfonamidopyrimidine (1.0 g, 4.25 mmol) was added portionwise. A mixture containing conc HNO_3 (0.35 g, 5.53 mmol) and 0.35 g of conc H_2SO_4 was added dropwise over a period of 30 min. Stirring was continued at 0°C for 1 hr then warmed slowly to room temperature. After 3 hours at room temperature, the viscous brown liquid was cooled to 0°C and poured over 50 g of cracked ice, resulting in the formation of a yellow precipitate. The product was collected by filtration yielding 0.71 g (59.7%) of yellow crystals of mp 216-217°C (literature mp 217-218°C). The nmr spectrum further supported the structural assignment as being 2-(m-nitrobenzenesulfonamido)pyrimidine.

Method C. A suspension of 2-benzenesulfonamidopyrimidine (1.0 g, 4.25 mmol) in 10 ml of acetic anhydride was cooled to 0°C and conc HNO_3 (0.35 g, 5.53 mmol) was added dropwise over a 15 minute period. Stirring was continued for 3 hr at 0°C then the solution was allowed to slowly warm to room temperature with agitation continuing for an additional 16 hours. The resulting yellow medium was poured into 100 ml of cold water with vigorous stirring which caused the deposition of a white precipitate. Filtration yielded 0.53 g (80.3%) of benzenesulfonamide mp 147-150°C (literature 150-151°C). Chilling of the filtrate returned 0.27 g (45.8%) of 2-hydroxy-5-nitropyrimidine: mp 202-204°C (literature[74] 203.5°C).

2-Benzenesulfonamido-5-iodopyrimidine (XVI)

This compound was prepared as described by English et al.[30] through iodination of 2-benzenesulfonamidopyrimidine with mercuric acetate and molecular iodine. The product was obtained in a 50.1% yield, recrystallization from glacial HOAc yielding white needles: mp 255-257°C (literature[32] 255-256°C).

2-Benzenesulfonamido-5-cyanopyrimidine (XXII)

A solution of 2-benzenesulfonamidopyrimidine (6.0 g, 16.7 mmol) in 15 ml of DMF was treated with cuprous cyanide (1.9 g, 21.2 mmol). The mixture was heated to 120°C and held at this temperature for 15 min. After rapid cooling to room temperature, the dark brown solution was poured into a mixture of 30 ml conc HCl and 15 g of ice. The brown granular solid which resulted was dissolved in 30 ml of cold conc NH_4OH and treated with zinc dust for approximately 1 min. After removal of the unreacted zinc dust, the solution was treated with Norit, then poured into a mixture containing 35 ml of 6N HCl and 15 ml HOAc. The pale brown precipitate which resulted was collected and recrystallized from HOAc yielding 3.78 g (87.9%) of pale yellow crystals: mp 226-227°C (literature[32] 226-227°C).

2-Benzenesulfonamido-5-carboxypyrimidine (XXIII)

A solution of 2-benzenesulfonamido-5-cyanopyrimidine (1.0 g, 3.86 mmol) in 10 ml of 10% aqueous KOH was refluxed for 1 hour. The hot solution was treated with Norit then cooled to

room temperature. Acidification with conc HCl resulted in formation of a white precipitate which was recrystallized from 1:1 EtOH: H_2O yielding 0.97 g (89.5%) of white needles: mp 285°C dec; ir (nujol) 3080 (sh, carboxyl OH) and 1715 cm^{-1} (C=O); nmr (DMSO-d_6) δ7.65 (m, 3H, 3Ar-H), 8.05 (m, 2H, 2Ar-H), 8.90 (s, 2H, pyrimidine-H) and 12.90 ppm (broad, 2H, NH, OH).

Anal. Calcd for $C_{11}H_9N_3O_4S$: C, 47.30; H, 3.25; N, 15.04

Found: C, 47.47; H, 3.39; N, 15.05

Attempted Preparation of 2-Benzenesulfonamido-5-carbethoxypyrimidine (XXIV)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (2.9 g, 10.4 mmol) in 50 ml of anhydrous EtOH was saturated with dry HCl and refluxed for 1.5 hr. After cooling to room temperature and addition of 10 ml of Et_2O to the medium, a white precipitate formed. Filtration yielded 2.61g (90%) of 2-benzenesulfonamido-5-carboxypyrimidine: mp 285°C dec.

2-Benzenesulfonamido-5-carbethoxypyrimidine (XXIV)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (2.0 g, 7.16 mmol) in 12 ml of thionyl chloride was refluxed for 4 hours. The residual thionyl chloride was removed *in vacuo* leaving a yellow solid. To this residue was added 25 ml of anhydrous EtOH and the resulting yellow solution was refluxed for 2 hours. An additional portion (25 ml) of EtOH was added and the yellow colored solution was treated with Norit. Sufficient water was added to the refluxing solution to induce cloudiness and with

slow cooling to room temperature, 1.8 g (81.7%) of white needles were deposited: mp 170.5-172°C ir (nujol), 3080 (NH) and 1720 cm^{-1} (ester C=O); nmr (DMSO-d_6) δ1.30 (t, 3H, C$\underline{H}_3$), 4.28 (m, 2H, O-C$\underline{H}_2$-), 7.66 (m, 3H, Ar-$\underline{H}$), 8.21 (m, 2H, Ar-H) and 8.96 ppm (s, 2H, pyrimidine-$\underline{H}$).

Anal. Calcd for $C_{13}H_{13}N_3O_4S$: C, 50.80; H, 4.26; N, 13.67
Found: C, 50.84; H, 4.38; N, 13.77

Attempted Preparation of 2-Benzenesulfonamido-5-carbohydrazinepyrimidine (XIX)

Hydrazine hydrate (0.25 g, 4.88 mmol) was added to 2-benzenesulfonamido-5-carbethoxypyrimidine in 50 ml of anhydrous EtOH and the resulting solution was refluxed for 14 hours. The white solid which remained after removal of the EtOH *in vacuo* was dissolved in a minimum amount of H_2O. When this solution was acidified with conc HCl, 0.61 g (67%) of 2-benzenesulfonamido-5-carboxypyrimidine was formed. The material was identical in all respects (ir and mp) to the authentic carboxylic acid prepared as described herein.

2-Benzenesulfonamido-5-carbohydrazinepyrimidine (XIX)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (1.0 g, 3.58 mmol) in 10 ml of thionyl chloride was refluxed for 4 hours. The resulting yellow solution was poured into 100 ml of dry CCl_4 and chilled in a refrigerator for 3 hours. The white precipitate which formed was collected by filtration and added cautiously to a solution of hydrazine hydrate (64% hydrazine

in H_2O) (2.0 ml, 2.06 g, 41.2 mmol) in 5 ml of H_2O. After stirring for 30 min, the mixture was diluted to 30 ml with H_2O. The clear solution was made slightly acidic with conc HCl, cooled in a refrigerator for 2 hours, and the white precipitate which formed was collected and washed with 20 ml of anhydrous EtOH followed by 20 ml of Et_2O. A yield of 0.63 g (60%) of white powder was obtained: mp turns yellow at 180°C, dec at 263°C; ir (nujol) 3620, 3280 (NH), 1655 and 1625 cm^{-1} (C=O); nmr ($DMSO-d_6$) δ 6.0 (broad, 5H, 2N$\underline{H}$, N$\underline{H}_2$ and 1/2 $\underline{H}_2O$), 7.70 (m, 3H, Ar-$\underline{H}$), 8.05 (m, 2H, Ar-$\underline{H}$) and 8.91 ppm (s, 2H, pyrimidine-$\underline{H}$).

Anal. Calcd for $C_{11}H_{11}N_5SO_3$ 1/2 H_2O: C, 43.70; H, 3.96; N, 23.16

Found: C, 44.00; H, 4.10; N, 23.16

2-Benzenesulfonamido-5-carbamylpyrimidine (XXI)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (1.0 g, 3.58 mmol) in 10 ml of thionyl chloride was refluxed for 3 hours. The excess thionyl chloride was evaporated *in vacuo* leaving a yellow solid which was added cautiously with stirring to 15 ml of conc NH_4OH. After stirring this mixture for 30 min at room temperature, the solution was evaporated to dryness *in vacuo* leaving a yellow solid which was dissolved in a minimum amount of water and treated with Norit. Upon acidification, a white precipitate formed which was recrystallized from EtOH/ H_2O yielding 0.91 g (91%) of white plates: mp 278° dec; ir (nujol) 3460, 3140 (NH), 1690 (amide C=O) and 1590 Cm^{-1} (amide II band);

nmr ($DMSO-d_6$) δ 7.68 (m, 3H, Ar-H), 8.02 (m, 2H, Ar-H), and 8.90 ppm (s, 2H, pyrimidine-H).

Anal. Calcd for $C_{11}H_{10}N_4SO_3$: C, 47.27; H, 3.62; N, 20.13

Found : C, 47.32; H, 3.74 N, 19.94.

2-Benzenesulfonamido-5-chlorocarbonylpyrimidine (XXV)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (1.0 g, 3.58 mmol) in 10 ml of thionyl chloride was refluxed for 4 hours. While the refluxing was continued, 100 ml of anhydrous CCl_4 was added. Upon slow cooling to room temperature, a white precipitate formed which was collected by filtration and washed with 3 x 10 ml portions of anhydrous Et_2O. After drying *in vacuo* for 14 hr a yield of 0.72 g (67%) of white solid was obtained: mp 207-208°C; ir (nujol) 1775 cm^{-1} (C=O); nmr (acetone-d_6) δ 7.65 (m, 3H, Ar-H), 8.08 (m, 2H, Ar-H) and 8.94 ppm (s, 2H, pyrimidine-H).

2-Benzenesulfonamido-5-(N-hydroxycarbamyl)pyrimidine (XX)

A suspension of 2-benzenesulfonamido-5-carboxypyrimidine (1.0 g, 3.58 mmol) in 10 ml of thionyl chloride was refluxed for 4 hr. Evaporation to dryness *in vacuo* left a yellow solid which was added cautiously to a solution of hydroxylamine hydrochloride (0.37 g, 5.32 mmol) and 5.5 ml of 1N NaOH (5.5 mmol) in 5 ml of H_2O. After stirring for 1 hr at room temperature, the yellow medium was acidified with conc HCl. The white

precipitate which formed was collected and recrystallized from 95% EtOH yielding 0.63 g (60%) of white crystals: mp 281° dec ir (nujol) 3340, 3250 (NH, OH) and 1650 cm^{-1} (C=O); nmr (DMSO-d_6) δ 7.65 (m, 3H, Ar-H), 8.05 (m, 2H, Ar-H) and 8.85 (s, 2H, pyrimidine-H), the OH and NH protons appear as a broad peak beneath the aromatic protons. A ferric chloride hydroxamic acid test was positive.

1-(2-Chloroethyl)-3-(D-glucos-2-yl) Urea (XLIII)

A solution of D-2-glucosamine hydrochloride) (5.35 g, 24.81 mmol) in 25 ml of 1N NaOH was treated with a solution of 2-chloroethyl isocyanate (2.62 g, 24.83 mmol) in 300 ml of 1:1 $CHCl_3Et_2O$. The heterogeneous suspension was stirred vigorously at 0°C for 3 hr and then agitated at room temperature for an additional 3 hr. During this time a white precipitate formed which was collected by filtration, and washed with 30 ml $CHCl_3$, 30 ml Et_2O and 30 ml pet ether (20-40°C). A yield of 5.08 g (75.6%) of white crystals was obtained: mp 150°C dec; ir (nujol) 3350 (OH) and 1630 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 6.40 (d, 1H, NH), 5.86 [s(broad, 1H, OH], 4.86 (s, IH, anomeric H), 4.35 (t, 1H, NH), remaining protons appear as a complex multiplet between 2.8 and 3.8 ppm.

Anal. Calcd for $C_9H_{17}N_2O_6Cl$: C, 37.97; H, 6.02; N, 9.84.

Found: C, 37.71; H, 5.99; N, 9.66.

2-Tetra-O-acetyl-D-glucosamine Hydrochloride (XXXIV)

This compound was prepared from D-2-glucosamine hydrochloride by the method of Bergmann and Zervas.[65] The product was obtained in an overall yield of 56.8%. Recrystallization from 95% EtOH gave white plates, mp 187-188°C (literature[65] 188°C).

1-(2-Chloroethyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XL)

Method A. A solution of 2-tetra-O-acetyl-D-glucosamine hydrochloride (3.0 g, 7.8 mmol) and sodium acetate (2.2 g, 18 mmol) in 100 ml of H_2O was extracted with 3 x 30 ml portions of $CHCl_3$. The combined extracts were dried over $MgSO_4$ for 1 hour. The drying agent was removed by filtration and the filtrate was treated with 2-chloroethyl isocyanate (0.85 g, 8.0 mmol). The clear solution was refluxed for 15 min and the $CHCl_3$ evaporated *in vacuo* leaving a white residue. Recrystallization of the solid from EtOH/H_2O yielded 2.40 g (75.3%) of white needles: mp 153-154°C; ir (nujol) 3320 (NH), 1740 (ester C=O) and 1630 cm^{-1} (urea C=O); nmr (acetone-d_6) δ 2.00 (m, 12H, $C\underline{H}_3$ C=O), 3.55 (m, 4H, N-$C\underline{H}_2$-$C\underline{H}_2$-Cl), 4.15 (m, 4H, $OC\underline{H}_2$, $OC\underline{H}$ and $NC\underline{H}$), 5.02 (m, 2H, O-$C\underline{H}$) and 5.85 ppm (m, 3H, [2N$\underline{H}$ and 1 O-C$\underline{H}$]).

Anal. Calcd for $C_{17}H_{25}N_2O_{10}Cl$: C, 45.09; H, 5.56; N, 6.19.

Found: C, 45.10; H, 5.62; N, 6.45.

Method B. 1-(2-Chloroethyl)-3-(D-glucos-2-yl) urea (3.0 g, 11.1 mmol) was suspended in acetic anhydride (10 ml, 10.82 g, 106 mmol) containing 20 ml of pyridine at 0°C. The mixture was stirred at 0° for 2 hr, then warmed slowly to room temperature with stirring continuing for 16 hrs. The resulting yellow medium was poured into 150 ml of cold water and chilled in an ice bath for 1 hr. The white precipitate was collected and recrystallized from EtOH/H_2O yielding 2.83 g (65%) of white needles: mp 153-154°C. Ir and nmr spectra were identical with the product obtained in Method A.

1-(2-Chloroethyl)-1-nitroso-3-(D-glucos-2-yl) Urea (XXXIXa)

A solution of 1-(2-chloroethyl)-3-(D-glucos-2-yl) urea 91.0 g, 3.69 mmol) and $NaNO_2$ (0.50 g, 7.38 mmol) in 30 ml of 2:1 H_2O:EtOH was treated dropwise with 2 ml of conc HCl at 0°C. The solution was stirred at 0°C for 30 min and then for 1 hr at room temperature. The clear yellow solution which resulted was cooled to 0°C and deposited 0.52 g of ivory colored crystals: mp 147-148°C dec with evolution of gas; ir (nujol) 3360 (OH), and 1690 cm^{-1} (nitroso urea C=O); nmr (DMSO-d_6) δ 7.80 (d, 1H, NH), 6.60 (s [broad], IH, OH), 5.15 (s[broad], IH, anomeric H), remaining protons appeared as a complex multiplex between 3.0 and 4.8 ppm.

Anal. Calcd for $C_9H_{16}N_3O_7Cl$: C, 34.46; H, 5.14

Found: C,34.59; H,5.30

1-(2-Chloroethyl)-1-nitroso-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLI)

Method A. A solution of 1-(2-chloroethyl)-3-(tetra-O-acetyl-D-glucos-2-yl) urea (0.5 g, 1.10 mmol) in 10 ml of HOAc at 15°C was treated with a solution $NaNO_2$ (0.085 g, 1.23 mmol) in 1.0 ml water. One ml of conc HCl was added and solution was stirred for 30 min. The yellow medium was poured into 100 ml of cold water and chilled in an ice bath for 2 hr during which time an ivory-colored precipitate formed. Recrystallization from $EtOH/H_2O$ yielded 0.33 g (63%) of ivory-colored needles: mp 141.5°C dec with evolution of gas; ir (nujol) 3400 (NH), 1740 (ester C=O) and 1710 cm^{-1} (nitroso urea C=O); nmr ($DMSO-d_6$) δ 2.00 (m, 12H, $C\underline{H}_3$-C=O), 3.65 (m, 4H, N-$C\underline{H}_2$-$C\underline{H}_2$-Cl), 4.15 (m, 4H, 1 O-C$\underline{H}$, 1N-C$\underline{H}$ and 1 OC$\underline{H}_2$), 5.25 (m, 2H, O-C$\underline{H}$), 5.95 (d, 1H, anomeric C$\underline{H}$), 8.95 ppm (d, 1H, N$\underline{H}$).

Anal. Calcd for $C_{17}H_{24}N_3O_{11}Cl$: C, 42.72; H, 4.99; N, 8.67.

Found: C, 42.74; H, 5.15; N, 8.67.

Method B. 1-(2-Chloroethyl)-1-nitroso-3-(D-glucos-2-yl) urea (3.29 g, 1.00 mmol) was suspended, pyridine at 0°C. The mixture was stirred at 0°C for 2 hr and then warmed to room temperature with stirring continuing for 16 hr. The resulting yellow solution was poured into 150 ml of cold water and cooled for 1 hr in an ice bath. The solid which formed was collected and recrystallized from $EtOH/H_2O$ yielding 1.9 g (36%) of pale yellow needles: mp 142°C dec with evolution of gas; ir and nmr spectra

were identical with those of the product of Method A.

1-(4-Methoxyphenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLIII)

A solution of 2-tetra-O-acetyl-D-glucosamine hydrochloride (2.0 g, 5.2 mmol) and sodium acetate (1.47 g, 11.99 mmol) in 65 ml of H_2O was extracted with 3 x 30 ml portions of $CHCl_3$. The combined extracts were dried over $MgSO_4$ for 1 hr. The drying agent was removed by filtration and the filtrate was treated with 4-methoxyphenyl isocyanate (0.80 g, 5.36 mmol). The clear solution was refluxed for 15 min and 50 ml of Et_2O added. Upon slow cooling, the solution deposited 2.34 g (91%) of white needles: mp 192-194°C; ir (nujol) 3300 (broad, NH), 1750 (ester C=O) and 1635 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 2.00 (m, 12H, $C\underline{H}_3$-C=O), 3.70 (s, 3H, O-$C\underline{H}_3$), 4.10 (m, 4H, $OC\underline{H}_2$, $OC\underline{H}$ and N-$C\underline{H}$), 5.15 (m, 2H, O-$C\underline{H}$), 5.85 (d, 1H, anomeric $\underline{H}$), 6.00 (d, 1H, $N\underline{H}$), 6.80 (d, 2H, Ar-$\underline{H}$). Recrystallization from EtOH/H_2O yielded fine white needles of mp 201-202.5°C: ir (nujol) 3300 (NH), 1750, 1730 (ester C=O) and 1665 cm^{-1} (urea C=O); nmr (DMSO-d_6) same as cited above. The ir spectra of both compounds in $CHCl_3$ were identical: 3420, 3360 (NH), 1745 (ester C=O) and 1665 cm^{-1} (urea C=O).

Anal. Calcd for $C_{22}H_{28}N_2O_{11}$: C, 53.22; H, 5.68; N, 5.64

Found: C, 53.19; H, 5.72; N, 5.56.

1-(2-Methoxyphenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLV)

A solution of 2-tetra-O-acetyl-D-glucosamine hydrochloride (2.0 g, 5.2 mmol) and sodium acetate (1.47 g, 11.99 mmol) in 65 ml H_2O was extracted with 3 x 30 ml portions of $CHCl_3$. The combined extracts were dried over anhydrous $MgSO_4$ for 1 hr. The drying agent was removed by filtration and the filtrate was treated with 2-methoxyphenyl isocyanate (0.80 g, 5.36 mmol). The clear solution was refluxed for 15 min and the $CHCl_3$ removed *in vacuo* leaving a white solid. Recrystallization from EtOH/H_2O yielded 2.14 g (83%) of white needles: mp 201.5-202°C; ir (nujol) 3380 (NH), 17.40 (ester C=O) and 1660 cm^{-1} (urea C=O); nmr (acetone-d_6) δ 2.00 (m, 12H, C$\underline{H}_3$ C=O), 3.80 (s, 3H, O-C$\underline{H}_3$), 4.15 (m, 4H, O-C$\underline{H}_2$, OC$\underline{H}$ and NC$\underline{H}$), 5.2 (m, 2H, O-C$\underline{H}$), 5.90 (d, 1H, NH), 6.95 (m, 3H, Ar-H), 7.70 (s, 1H, NH) and 8.25 ppm (m, 1H, Ar-H).

Anal. Calcd for $C_{22}H_{28}N_2O_{11}$: C, 53.22; H, 5.68; N, 5.64

Found: C, 53.15; H, 5.70; N, 5.46

1-(2-Ethoxyphenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLVI)

A solution of 2-tetra-O-acetyl-D-glucosamine hydrochloride (2.0 g, 5.2 mmol) and sodium acetate (1.47 g, 11.99 mmol) in 65 ml of water was extracted with 3 x 30 ml portions of $CHCl_3$. The combined extracts were dried over $MgSO_4$ for 1 hour. After the drying agent was removed by filtration the filtrate was treated with 2-ethoxyphenyl isocyanate (0.87 g, 5.36 mmol) and the clear solution which resulted was refluxed for 15 min. The $CHCl_3$ was removed *in vacuo* leaving a white solid. Recrystallization from EtOH/H_2O yielded 2.3 g (87%) of white needles: mp 202-203°C;

ir (nujol) 3310 (NH), 1750 (ester C=O) and 1640 cm^{-1} 2.00 (m, 12H, $C\underline{H}_3$-C=O), 4.05 (m, 6H, 20-$C\underline{H}_2$, O-C$\underline{H}$ and N-C$\underline{H}$), 5.17 (m, 2H, O-C$\underline{H}$), 5.84 (d, 1H, anomeric $\underline{H}$), 6.31 (d, 1H, N$\underline{H}$), 6.87 (m, 3H, Ar-$\underline{H}$), 7.63 (s, 1H N$\underline{H}$) and 8.28 ppm (m, 1H, Ar-$\underline{N}$).

Anal. Calcd for $C_{23}H_{30}N_2O_{11}$: C, 54.11; H, 5.92; N, 5.49

Found: C, 54.07; H, 5.90; N, 5.34

Preparation of 2-Tetra-O-acetyl-D-glucosamine

This compound was prepared in 87% yield by the method of Bergmann and Zervass, mp 143-144°C (literature[75] 143°C).

1-(3-Methoxyphenyl0-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLIV)

A solution of 2-tetra-O-acetyl-D-glucosamine (2.0 g, 5.76 mmol) in 20 ml of $CHCl_3$ was treated with 3-methoxyphenyl isocyanate (0.86 g, 5.76 mmol). The colorless solution was refluxed for 15 min then evaporated to dryness *in vacuo* leaving a yellow oil which was triturated with 5 ml of anhydrous EtOH to induce crystallization. The white solid was collected and recrystallized from EtOH/H_2O yielding 2.49 g (87.1%) of white needles: mp 202-203°C; ir (nujol) 3370 (NH), 1740 (ester C=O) and 1670 cm^{-1} (urea C=O); nmr (acetone-d_6) δ 2.0 (m, 12H, $C\underline{H}_3$-C=O), 3.75 (s, 3H, O-$C\underline{H}_3$), 4.20 (m, 4H, OCH_2, OC$\underline{H}$ and NC$\underline{H}$), 5.25 (m, 2H, 2 O-C$\underline{H}$), 5.92 (d, 1H, anomeric $\underline{H}$), 6.60 (m, 2H, N$\underline{H}$),7.20 (m, 3H, Ar-$\underline{H}$) and 8.05 ppm (s, 1H, Ar-$\underline{N}$).

Anal. Calcd for $C_{22}H_{28}N_2O_{11}$: C, 53.22; H, 5.68; N, 5.64

Found: C, 53.48; H, 5.70; N, 5.60

1-(4-Tolyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea XLVII

A solution of 2-tetra-O-acetyl-D-glucosamine hydrochloride (2.0 g, 5.2 mmol) and sodium acetate (1.47 g, 11.99 mmol) in 65 ml of H_2O was extracted with 3 x 30 ml portions of $CHCl_3$. The combined extracts were dried over anhydrous $MgSO_4$ for 1 hr. The drying agent was removed by filtration and the filtrate was treated with 4-tolyl isocyanate (0.71 g, 5.33 mmol). The clear solution was refluxed for 15 min and the $CHCl_3$ evaporated *in vacuo* leaving a yellow amorphous solid. Recrystallization from EtOH/H_2O yielded 2.22 g (89.2%) of white needles: mp 196-197°C; ir (nujol) 3380, 3340 (NH), 1745, 1725 (ester C=O) and 1670 cm^{-1} (urea C=O); nmr (acetone-d_6) δ 2.00 (m, 12H, $C\underline{H}_3$-C=O), 2.22 (s, 3H, Ar-$C\underline{H}_3$), 4.15 (m, 4H, 1 O-$C\underline{H}_2$, 1 O-C$\underline{H}$, 1 N-C$\underline{H}$), 5.25 (m, 2H, O-C$\underline{H}$), 5.90 (d, 2H, 1N$\underline{H}$ and anomeric proton), 7.08 (d, 2H, Ar-$\underline{H}$), 7.30 (d, 2H, Ar-H) and 7.90 ppm (s, 1H, NH).

Anal. Calcd for $C_{22}H_{28}N_2O_{10}$: C, 54.99; H, 5.87; N, 5.83

Found: C, 54.99; H, 5.88; N, 5.55

1-Phenyl-3-(tetra-O-acetyl-D-glucos-2-yl) Urea(XLVIII)

This compound was prepared in a 91% yield by the method of Micheel and Lengsfeld.[75] Recrystallization from EtOH/H_2O gave long white needles of mp 203.5-204°C (literature[75] 204°C): ir (nujol) 3380 (NH), 1745, 1725 (ester C=O) and 1665 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 2.00 (m, 12H, $C\underline{H}_3$-C=O), 4.08 (m, 4H, 1 O$C\underline{H}_2$, 1 O-C$\underline{H}$ and 1 N-C$\underline{H}$), 5.20 (m, 2H, O-C$\underline{H}$), 5.88 (d, 1H, anomeric $\underline{H}$), 6.10 (d, 1H, N$\underline{H}$), 7.30 (m, 5H, Ar-$\underline{H}$) and 8.60 ppm (s, 1H, N$\underline{H}$).

3,4,6-(Tri-O-acetyl-D-glucopyrano)-1,2,d-[3-(4-methoxyphenyl)] imidazolidine-2-one (LIII)

A solution of glucosamine (1.0 g, 5.58 mmol) in 10 ml pyridine was treated with 4-methoxy-phenyl isocyanate (0.83 g, 5.58 mmol). The solution was stirred for 30 min at room temperature then heated to 40°C for 1 hr. The yellow colored medium was cooled to 0°C and acetic anhydride (5 ml [5.42 g], 53.09 mmol) was added dropwise over a period of 30 min. The solution was stirred at 0°C for 1 hr then warmed slowly to room temperature with stirring continuing for 16 hr. After cooling to 0°, 100 ml of H_2O were added. The white precipitate which resulted was collected and recrystallized from anhydrous ethanol yielding 0.77 g (31.5%) of white needles: mp 192-4°C; ir (nujol) 3380, 3320 (NH), 1750, 1725 (ester C=O), 1665 and 1640 cm^{-1} (imidazole C=O); nmr (acetone-d_6) δ 2.05 (m, 9H, $C\underline{H}_3$-C=O), 3.80 (s, 3H, O-$C\underline{H}_3$), 4.20 (m, 4H, O-$C\underline{H}_2$, O-C$\underline{H}$, N-C$\underline{H}$), 5.25 (m, 2H, O-C$\underline{H}$), 6.95 (d, 2H, Ar-$\underline{H}$) and 7.4 (d, 2H, Ar-$\underline{H}$).

Anal. Calcd for $C_2H_{24}N_2O_9$: C, 55.04; H, 5.54; N, 6.42

Found: C, 54.89; H, 5.79; N, 6.18.

1-(4-Methoxy-3-nitrosophenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (LVI)

A solution of 1-(4-methoxyphenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) urea (0.5 g, 1.0 mmol) in 10 ml HOAc at 15°C was treated with $NaNO_2$ (0.10 g, 1.5 mmol, in 1.0 ml H_2O). One ml of conc HCl was added to the yellow colored medium, causing a bright

yellow color to form. Stirring was continued for 90 min at room temperature then 100 ml of cold H_2O was added. After cooling to 0°, the yellow precipitate was collected and recrystallized from EtOH/ H_2O yielding 0.33 g (62.9%) of small yellow needles: mp 160-162°C; ir (nujol) 3320, 3280 (NH), 1750 (sh), 1740 (ester C=O) and 1640 cm^{-1} (urea C=O), nmr (acetone-d_6) δ 2.00 (m, 12H, C$\underline{H}_3$-C=O), 3.88 (s, 3H, O-C$\underline{H}_3$) 4.12 (m, 4H, O-C$\underline{H}_2$ N-C$\underline{H}$, O-C$\underline{H}$), 5.20 (m, 2H, 2 O-C$\underline{H}$), 5.90 (d, 1H, anomeric $\underline{H}$), 6.90 (s[broad], 1H, N$\underline{H}$), 7.40 (m, 2H, Ar-$\underline{H}$), 8.32 (d, 1H, Ar-$\underline{H}$) and 9.18 ppm (s[broad], 1H, N$\underline{H}$).

Anal. Calcd for $C_{22}H_2N_3O_{12}$ H_2O: C, 48.62; N, 5.37; N, 7.73.

Found: C, 48.35; H, 5.02; N, 7.73.

1-(4-Nitrophenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (XLIX)

A solution of tetra-O-acetyl-D-glucosamine (1.0 g, 2.88 mmol) in 100 ml $CHCl_3$ was treated with 4-nitrophenyl isocyanate (0.47 g, 2.88 mmol) in 50 ml of $CHCl_3$. After stirring for 5 min, the entire mass gelled. The gel was held for 1 hr at room temperature, then suction filtered to remove a yellow solid. The product was washed first with cold anhydrous EtOH then with Et_2O. The yellow powder was dissolved in boiling 95% EtOH and treated with Norit. Upon slow cooling, the solution again gelled but filtration yielded 1.38 g (93.9%) of pale yellow solid: mp discolors at 215°C and dec at 222°C; ir (nujol) 3360 (NH), 1740 (ester C=O) and 1680 cm^{-1} (urea C=O); nmr (DMSO-d_6/acetone-d_6) δ 2.0 (m, 12H, C$\underline{H}_3$-C=O); 4.15 (m, 4H, O-C$\underline{H}_2$, OC$\underline{H}$ and NC$\underline{H}$); 5.90 (m, 2H, 2 O-C$\underline{H}$);

5.92 (d, 1H, anomeric H); 6.45 (d, 1H, NH); 7.78 (d, 2H, Ar-H), 8.10 (d, 2H, Ar-H) and 9.35 ppm (s, 1H, NH).

Anal. Calcd for $C_{21}H_{25}N_3O_{12}$: C, 49.32; H, 4.93; N, 8.22
Found: C, 49.19; H, 4.97; N, 7.96

1-(4-Anilino)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (LI)

A solution of 1-(4-nitrophenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) urea (1.0 g, 1.96 mmol) in 150 ml MeOH containing approximately 0.2 g 10% Pd on charcoal was hydrogenated in a Parr apparatus for 20 hr at a H_2 pressure of 50 psi. The catalyst was removed by filtration and the colorless filtrate was evaporated to dryness *in vacuo* leaving a gray solid. Recrystallization from 1:1 Et_2O/pet ether (20-40°C) yielded 0.89 g (94.5%) of small white crystals: mp discolors at 204° and dec at 208.5°C; ir (nujol) 3410, 3320 (NH), 1740 (ester C=O) and 1660 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 2.00 (m, 12H, CH_3 C=O); 4.08 (m, 4H, O-CH_2, OCH and NCH); 4.98 (m, 4N, 2 O-CH and 2 NH); 5.80 (m 2H, anomeric H and NH); 6.45 (d, 2H, Ar-H), 6.95 (d, 2H, Ar-H) and 8.08 ppm (s[broad], 1H, NH).

Anal. Calcd for $C_{21}H_{27}N_3O_{10}$: C, 52.39; H, 5.65; N, 8.73
Found: C, 52.26; H, 5.83; N, 8.54.

1-(3-Nitrophenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (L)

Tetra-O-acetyl-D-glucosamine (1.0 g, 2.88 mmol) was added in small portions to a solution of 3-nitrophenyl isocyanate (0.47 g, 2.88 mmol) in 10 ml of $CHCl_3$ at room temperature. The mixture was refluxed for 15 min and upon addition of 5 ml of Et_2O, the

entire contents solidified. After standing for 1 hr at room temperature, the brown precipitate was collected and washed with Et_2O and pet ether (20-40°C). Recrystallization from anhydrous EtOH yielded 1.3 g of white needles: mp 198°C; ir (nujol) 3350, 3270 (NH), 1745 (ester C=O) and 1650 cm^{-1} (urea C=O); nmr (DMSO-d_6/acetone-d_6) δ 2.05 (m, 12H, $C\underline{H}_3$-C=O); 4.17 (m, 4H, O-$C\underline{H}_2$, NC$\underline{H}$ and OC$\underline{H}$); 5.30 (m, 2H, 2 OC$\underline{H}$); 5.92 (d, 1H, anomeric $\underline{H}$), 6.40 (d, 1H, N$\underline{H}$), 7.73 (m, 3H, Ar-$\underline{H}$), 8.55 (t, 1H, Ar-$\underline{H}$) and 9.23 ppm (s, 1H, N$\underline{H}$).

Anal. Calcd for $C_{21}H_{25}N_3O_{12}$: C, 49.32; H, 4.93; N, 8.22
Found: C, 49.28; H, 5.00; N. 8.03

1-(3-Anilino)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (LII)

A solution of 1-(3-nitrophenyl)-3-(tetra-O-acetyl-D-glucos-2-yl) urea (1.0 g, 1.96 mm) in 150 ml of MeOH containing approximately 0.2 g of 10% Pd on charcoal was hydrogenated in a Parr apparatus for 20 hr at a H_2 pressure of 50 psi. The catalyst was removed by filtration and the clear filtrate was evaporated to dryness *in vacuo* leaving a gray solid. Recrystallization from absolute EtOH yielded 0.82 g (87.2%) of small white needles: mp 200.5°C dec; ir (nujol) 3460, 3370, 3320 (NH), 1750, 1730 (ester C=O) and 1675 cm^{-1} (urea C=O); nmr (DMSO-d_6) 2.00 (m, 12H, 4$C\underline{H}_3$-C=O), 4.08 (m, 4H, O-$C\underline{H}_2$, NC$\underline{H}$ and O-C$\underline{H}$), 4.95 (m, 4H, 2 OC$\underline{H}$ and $N\underline{H}_2$), 5.70 to 7.00 (complex multiplet, 6H, 4 Ar-$\underline{H}$, NH and anomeric $\underline{H}$ and 8.3 ppm (s, 1H, N$\underline{H}$).

Anal. Calcd for $C_{21}H_{27}N_3O_{10}$: C, 52.39; H, 5.65; N, 8.73
Found: C, 52.21; H, 5.75; N, 8.45

1-(p-Toluenesulfonyl)-3-(tetra-O-acetyl-D-glucos-2-yl) Urea (LVII)

A solution of 2-tetra-O-acetyl-D-glucosamine (3.0 g, 8.64 mmol) in 150 ml of $CHCl_3$ was cooled to)°C and p-toluenesulfonyl isocyanate (1.70 g, 8.64 mmol) in 15 ml of $CHCl_3$ was added dropwise. Stirring was continued for 1 hr at room temperature, whereafter the $CHCl_3$ was removed *in vacuo* leaving a white solid. Recrystallization from anhydrous EtOH yielded 4.28 g (91.%) of fine white needles: mp discolors at 199°C dec at 213°C; ir (nujol) 3260, 3170 (NH), 1745 (ester C=O) 1700 (C=O) and 1660 cm^{-1} (urea C=O); nmr (DMSO-d_6) δ 1.72 (s, 3H, $C\underline{H}_3$ C=O), 2.38 (s, 3H, Ar-$C\underline{H}_3$), 4.03 (m, 4H, O-$C\underline{H}_2$, O-C$\underline{H}$ and NC$\underline{H}$), 5.09 (m, 2H, 2 OC$\underline{H}$), 5.75 (a, 1H, anomeric $\underline{H}$), 6.42 (m, 2H, 2N$\underline{H}$), 7.42 (d, 2H, Ar-$\underline{H}$) and 7.70 ppm (d, 2H, Ar-$\underline{H}$).

Anal. Calcd for $C_{22}H_{28}N_2SO_{12}$: C, 48.53; H, 5.18; N, 5.14
Found: C, 48.35; H, 5.26; N,4.97

Part II: Preparation of Labeled Compounds

Ferrous Sulfate Procedure

The desired amount of pertechnetate solution (2 to 10 mCi) from the Technetope Generator was diluted to 2 ml with saline and $FeSO_4$ $7H_2O$ (10 mg, 3.6 x 10^{-2} mmol) was added. After stirring for 5 min, 1 ml of 0.4 m ligand solution was added and the pH adjusted to 5 with 3N NaOH. Following the addition of 2 ml of 0.1 m KH_2PO_4 the pH was raised to 7 with 1N NaOH. At this point, if the solution was not homogeneous, it was filtered (with suction) through Whatman paper #1. The filtrate (a maximum of 3 ml) was placed on a Sephadex G25-m column (preparation described below) and eluted with saline. A 10 ml aliquot was taken from the most active portion of the chelate fraction and sterilized by filtration through a sterile millipore filter directly into a sterile, pyrogen free evacuated vial.

Preparation of Sephadex Column

A suspension of 7.0 g of Sephadex G25-M beads in 250 ml of distilled water was stirred gently for 24 hours. This suspension was used to prepare a column (1.2 x *ca*. 30 cm) in a 100 ml graduated burette. The column was flushed with 50 ml of saline prior to use. After each use, the column was saturated with formalin to prevent microbial decomposition of the resin. This formalin was washed from the column with a minimum of 50 ml of saline prior to reuse.

Stannous Chloride Procedure

The desired amount of pertechnetate solution (2 to 10 mCi) from the Technetope Generator was diluted to 2 ml with saline. After the addition of 0.5 ml of stock stannous chloride solution (prepared by dissolving 20 mg $SnCl_2$ $2H_2O$ in 2 ml of conc HCl followed by dilution to 10 ml with saline), the pH was lowered to 1.5 with conc HCl. After stirring for 1 min, the pH was adjusted to 2.5-3.0 with 1 N NaOH and 1.0 ml of 0.4 m ligand solution added. A 3.0 ml aliquot of this solution was purified as described under the ferrous sulfate procedure.

Stannous Chloride Procedure B

This method was essentially the same as described in Procedure A, with the exception of the final pH. Prior to gel filtration, the pH was raised to 8 by the addition of NaOH (see ref 36 for details).

Preparation of ^{131}I-Labeled Compounds

The procedures used to label compounds (XL, XLI, XLIII and XXXIXa) are described in detail in reference 36a.

Tissue Distribution Studies

The experimental animal* was anesthesized with ether

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* Initially, an outbred strain of albino Wistar rats of both sexes was utilized, while later studies were performed only on males of an inbred strain of albino Fisher rats. In all cases, the weight of the rats ranged between 120 and 300 g.

and the radiopharmaceutical (0.3 to 0.5 ml of solution prepared as described above) was injected into the tail vein. A good injection showed no activity in the tail and high activity in the heart (monitored with a Geiger-Müller tube) immediately after injection. The animals were weighed to the nearest 0.1 g and sacrificed at the times specified by asphixiation in a dessicator saturated with ether vapor. Immediately before death, a cardia puncture was performed and 1.0 ml of blood withdrawn. The animals were autopsied removing the desired organs which were dissected from fat and weighed to the nearest 0.0001 g. Each tissue sample was counted for 1 min in a well counter.

The activity in the standard injection dose was determined by diluting the injection dose to 100 ml and counting 1 ml of this solution in the well counter for 1 min. This value corresponds to 1% of the activity in the injection dose and was used as a standard. The percent dose per gram (% dose/g) values were calculated as follows: (1) The background activity, counts per min (cpm), was subtracted from cpm for each organ. (2) Cpm-BG was divided by the weight of the organ counted yielding (cpm-BG)/g. (3) (Cpm-BG)/g was divided by cpm of the standard yielding % dose/g. (4) This value was then normalized as described earlier.

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VITA

H. Donald Burns, the son of George D. and Betty M. Burns, was born April 17, 1946, in Scranton, Pennsylvania

He received his elementary education in the public school of Scranton, Pennsylvania, and graduated in 1964 from West Scranton Senior High School.

He received his undergraduate training at the University of Scranton, where he received a bachelor of science degree in 1968.

Mr. Burns entered Lehigh University as a graduate teaching assistant in September, 1970, and received a master of science degree in June of 1972.

Mr. Burns is a member of the American Chemical Society and the Society of Sigma Xi.