RADIOPHARMACOLOGY OF IMINODIACETIC ACID N-DERIVATIVES ANALYSIS IN BIOLOGICAL MODEL AND COMPARISON TO HUMAN BEINGS.

C.O.CANELLAS, M.G.ARGUELLES AND A.E.A.MITTA

COMISION NACIONAL DE ENERGIA ATOMICA DE LA REPUBLICA ARGENTINA.

BUENOS AIRES
REPUBLICA ARGENTINA
1987

RADIOPHARMACOLOGY OF IMINODIACETIC ACID N-DERIVATIVES ANALYSIS IN BIOLOGICAL MODELS AND COMPARISON TO HUMAN BEINGS

Cañellas.C.O; Argüelles.M.G and Mitta A.E.A
National Atomic Energy Commission
Research Subprogram, Application and Development in Nuclear Medicine. Buenos Aires, Argentina.

INTRODUCTION

From the time Harvey, Burke and Halko published their first reports on images of the liver and biliary tract using "Bengal Rose" labeled with I-131, the diagnostic file on liver and gallbladder disorders has grown considerably. In addition to the wealth of information obtained over the years from contrastive radiology with iodates, new methodology has provided us with more than just functional information.

The body processes involved in the conveyance of these chelates with hepatobiliary affinity can be summed up as follows:

1.- the radiopharmaceutical rapidly binds with the plasmatic carrier and reaches the hepatocyte's bloodstream.
2.- its passage through the hepatocyte and elimination through the biliary ducts.
3.- passage through intrahepatic ducts to gallbladder.
4.- passage through the choledochus to the duodenum.

Keeping in mind the above and that a pharmaceutical that goes through these steps, in order to prove useful, must have the properties of rapid plasmatic clearing, high hepatic uptake with an effective flow to the gallbladder regardless of the tenor of the nonconjugated billirubin present, and very low kidney excretion, we decided to make a complete radiopharmacological study of a series of iminodiacetic acid N-derivatives, employing not only novel methods of synthesis but also reliable chromatographic systems as well as a biological model that would allow us to extrapolate from the experimental results to apply them to humans. On the basis of

the data obtained from the four derivatives used in the experiment, we were able to design a new derivative whose biological behavior in human beings we were able to predict and which we verified on normal volunteers and carriers of different clinicopathologic diseases.

MATERIALS AND METHODS

The method published by Harvey et al was followed to obtain the N-derivatives. Modifications were introduced to improve the efficiency of the intermediate as well as end product reactions.

The following four compounds were synthesized using the synthetic pathway as seen in figure 1:
N-(2,6 dimethylphenylcarbamoylmethyl)iminodiacetic acid, N-(2,6 diethylphenylcarbamoylmethyl)iminodiacetic acid, N-(2,6 diisopropylphenylcarbamoylmethyl)iminodiacetic acid and N-(4, butylphenylcarbamoylmethyl)iminodiacetic acid.

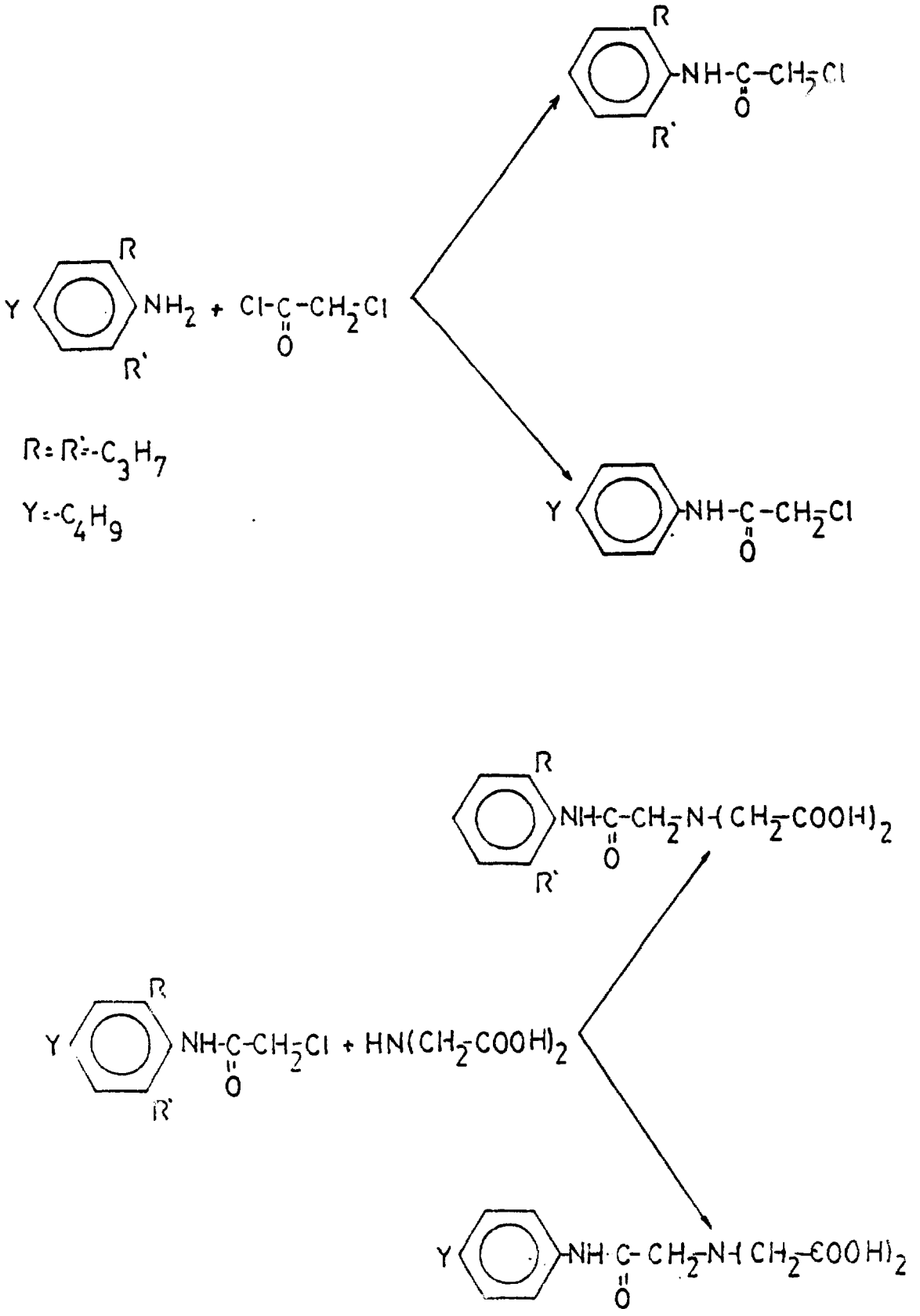
Also, the previously unknown N-derivative, N-(2,6 diisopropylphenylcarbamoylethyl 1)iminodiacetic acid was obtained by way of the synthetic pathway seen in figure 2.

The quality control methodology chosen was the determination of melting point, with a Kofler device, spectra of nuclear magnetic resonance at 100 mHz a Varian XL-110-15 spectrophotometer in a solution of DMS(H-3) with tetramethylsilene as an internal pattern, mass spectrometry at 70 eV on a Varian Mat-CH 7A spectrometer run by a Varian-Mat-Data system 166 computer with direct sample insertion and determination of the centesimal formula.

We used $Cl_2Sn.2\ H_2O$ as a reducing agent and sodium pertechnetate (Tc-99m) from a "Gentec" generator. Each vial contained 20.0 mg of the N-derivative and 0.20 mg of the reducing agent, kept in a nitrogen atmosphere at a volume of 1.0 ml, to which 20 mCi (740 MBq) sodium pertechnetate (Tc-99m) was added resulting in a final volume of 2.0 ml.

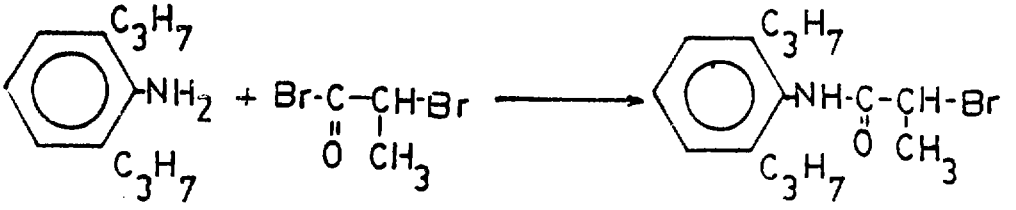
Given the fact that there are no reliable chromatographic systems to correlate the chelate's radiochemical purity and

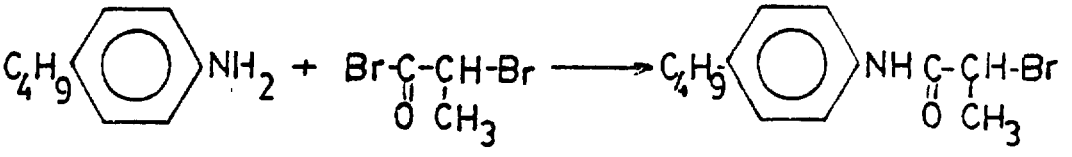
figure 1

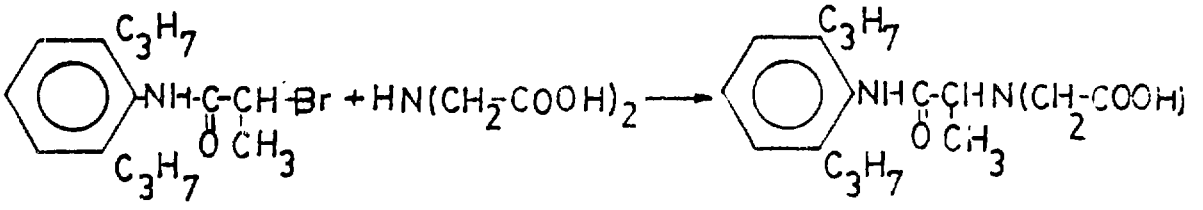


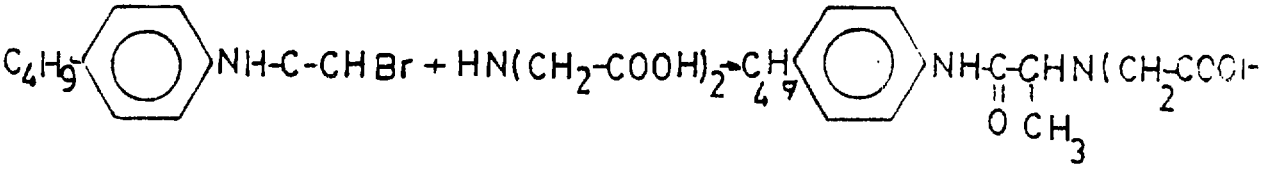
SYNTHESIS OF N-DERIVATIVES

Figure 2









biodistribution, we found it necessary to develop them. With ITLC(SG) as a base and methanol 85% we differentiated the colloid from the bis-compound as the former remained in the base while the latter ran with the solvent front, the same as the free pertechnetate.

By altering the polarity of the solvent mixture, we were able to inhibit the migration of the N-derivatives while the free pertechnetate remained Rf 1.0; the composition of the solvent was methylethylketone (MEK):benzene:methanol (1.0: 8.5:0.5). We also employed molecular screen (sephadex G-25 medium) stacked in glass tubes. After preparation, the dead volume was eluted with a physiological solution and the distribution of the activity in the tube was determined with a Berthold LB 2723 radiochromatograph at a speed of 3000 mm/h.

The lethal dose 50 (LD-50) was determined for each one of the compounds to deduce the mass to be injected without causing toxic reactions, and the safety factor, calculated. Acute toxicity and sterility tests were run in accordance with Argentine National Pharmacopoeia (6th edition) methodology. To determine the presence of pyrogenic substances, not only was the standard technique of basal temperature variation on rabbits used but also the "limulus test", recently accepted by the US Pharmacopoeia.

Since the speed of the distribution in the tissues of each organ will be determined by the bloodstream and by the ease with which the molecules can pass through the bloodstream's capillary network and penetrate the cells, it was necessary to employ a technique that would enable us to evaluate plasmatic kinetics and thus determine the "plasmatic half life" indicated by the duration and intesity of the desired effect of each one of the radiopharmaceuticals, being able to infer its behavior. We used Wistar rats for this and performed a carotidea-carotideana bypass on them by inserting a plastic cannula with mandrel, after divulsion and b[illegible] cutting the right jugular vein (figure 3), and a 1.0 m long catheter with a 0.7 mm internal diameter in the left primitive carotid artery, in order to attain extracorporeal circulation (figure 4)

figure 3

figure 4

We placed the animal near an Alfanuclear MOS single channel gamma spectrometer with a detector containing a NaI(Tl) crystal, 1 3/4" wide by 2" high, and inserted the carotid catheter. 50 μCi of radiopharmaceutical was administered via the jugular plasmatic cannula and the variation in circulating radioactivity was recorded up to 120 minutes; by recording the results on semilogarithmic graph paper we arrived at the value of the components, the function, which gave us an idea of the velocity of hepatic uptake and renal elimination.

The biodistribution of the five radiopharmaceuticals was estimated in mice (NIH strain) administering them 0.1 ml of the chelate via the tail vein, then distributing them in metabolic boxes and sacrificing them at different times by cervical traction. The abdominal cavity was opened (fig. 5), the choledochus pinched, and the gallbladder removed without spilling its contents, then the liver, spleen, duodenum, intestines, kidneys, bladder and urine (fig 6). By using a Carrintec CR-30 ionization chamber run by a CRV-PV computer, the residual activity was determined in each one of the organs and with the resulting figures the percentage curves of the injected dose is function of time were drawn.

We considered two elimination routes, the biliary and the renal, and by reinjecting the biliary contents and urine, we were able to evaluate the metabolic influence of each of these paths. The gallbladder were removed from mice that had been injected with different radiopharmaceuticals and the urine was collected in a metabolic box. These were measured in an automatic gamma counter and they were administered to the same biological model as was used in the biodistribution. Afterwards, the animals were sacrificed at different times and the residual radioactivity dose in each organ was determined. The results obtained were compared to the standard ones by the matching "t" test.

Since one of the objectives of this project was the synthesis and biological evaluation of a new N-derivative

figure 5

Figure 6

and the subsequent extrapolation from the results in the biological model use on human beings, tests were carried out in different branches of nuclear medicine, on normal volunteers and carriers of different clinicopathologic disorders. We used the new compound and, given its structural likeness to N-(2,6 diisopropylphenylcarbamoylmethyl)imino-diacetic acid, matching tests were performed. Eleven normal volunteers were compared who were administered the drug a week apart. Twenty nine adult patients were divided into two clinicopathologic groups: acute and chronic cholecystitis, and were given the N-(2,6 diisopropylphenylcarbamoylethyl 1) iminodiacetic acid. The new derivative was tested on 30 patients divided into four clinicopathologic groups: acute cholecystitis, chronic cholecystitis, cirrhosis and jaundice.

The takes were done uninterruptedly using a gamma camera, an image every minute for 35 minutes, followed by one every 15 minutes. By creating areas of interest in the heart and the liver, we obtained the liver/heart activity radio at 5 and 60 minutes. Also, we estimated the appearance times of the intrahepatic ducts (IAT), extrahepatic ducts (EAT), gallbladder (GAT), duodenum (DAT), and renal persistence (RP).

RESULTS

Using the methodology described above, the following reaction efficiencies were obtained: N-(2,6 dimethylphenyl-carbamoylmethyl)iminodiacetic acid (75%), N-(2,6 diethylphenyl-carbamoylmethyl)iminodiacetic acid (80%), N-(2,6 diisopropyl-phenylcarbamoylmethyl)iminodiacetic acid (75%), N-(4 butyl-phenylcarbamoylmethyl)iminodiacetic acid (90%) and N-(2,6 diisopropylphenylcarbamoylethyl 1)iminodiacetic acid (75%). The mass spectra, nuclear magnetic resonance and centesimal composition corresponded to the proposed structures.

The LD-50 arrived at were 130±5.8 mg/Kg for dimethyl derivative, 160±4.2 mg/Kg for the diethyl derivative, 150±6.3 mg/Kg for the diisopropyl derivative, 72±2.4 mg/Kg for the

p-butyl derivative, and 140±6.2 mg/Kg for the new derivative. With respect to these data, the following safety factors were obtained: 456 (dimethyl derivative), 526 (diethyl derivative), 561 (diisopropyl derivative), 252 (p-butyl derivative) and 491 (new derivative).

No pyrogenous substances were found and the limulus test proves to be not only totally compatible with these radiopharmaceuticals but also homologous with the Argentine National Pharmacopoeia.

Using the carotidea-carotideana bypass technique, we determined that the blood mixing time for all compounds is 20 seconds, with no significant differences; the T½ short (hepatic uptake) and T½ long (renal excretion) were 2.5 and 92 minutes for the dimethyl derivative, 3.0 and 116 minutes for the diethyl derivative, 2.8 and 130 minutes for the diisopropyl derivative, 2.0 and 238 minutes for the p-butyl derivative and 2.0 and 136 minutes for the new derivative.

With the figures obtained in the biodistribution, variation graphs in function of time were drawn up of the injected dose for renal excretion, intestinal passage, hepatic clearing and biliary uptake. On the basis of these data, we determined the maximum uptake time in the bladder to be 5 minutes for the dimethyl derivative, 30 minutes for the diethyl derivative, 15 minutes for the diisopropyl derivative, 30 minutes for the p-butyl derivative and 15 minutes for the new derivative.

In using the reinjection technique, we found that when bile was administered, a similar uptake by the bladder occurred on the whole, staying above normal values of renal elimination. When urine was administered, we observed a completely different biological behavior: bladder uptake was practically nil and renal elimination, much higher than normal levels.

In normal volunteers, treated with the diisopropyl derivative, we observed an IAT of 7.4±1.4 minutes, an EAT of

12.7±2.7 minutes, a GAT of 12.9±2.7 minutes, a DAT of 24.2±4.6 minutes and RP of 8.8±3.8 minutes. For the new derivative, these values were 7.3±2.1 minutes, 11.6±3.4 minutes, 13.7±4.7 minutes, 21.3±7.0 minutes and 10.5±6.8 minutes, respectively.

Among the acute cholecystitis cases, the results were and IAT of 11.4±3.9 minutes and 10.5±3.3 minutes, an EAT of 22.0±4.8 minutes and 16.5±6.5 minutes for de diisopropyl derivative and for the new radiopharmaceutical, respectively. It was not possible to observe the gallbladder in any of the tests run.

In the cases of chronic cholecystitis, the results were an IAT of 9.4±2.6 minutes and 6.7±2.5 minutes, an EAT of 16.7±7.3 minutes and 12.4±4.3 minutes for the diisopropyl derivative and the new derivative, respectively. In the tests run with diisopropyl derivative, the gallbladder did not appear, while with the new derivative, it was detected in three of the patients.

DISCUSSION

Since the appearance of the dimethyl derivative, many more compounds have appeared. In this study, we have used only some of them, not because they could not be synthesized, but because they did not seem relevant to our objectives.

Our first objective was to achieve a greater reaction efficiency in the methods proposed by Harvey *et al.* The changes introduced improved the reaction by 75-90%, depending on the derivative. This improvement made possible the synthesis of a new compound in which we modified two of the variables affecting the behavior of these chelates, the molecular weight, and lipophilic power; to this effect, we worked on modifying the hydrocarbonate unit that separates the hydrophilic and lipophilic nuclei. To achieve an optimum control of it, we neede to develop radiochemical techniques that would truly reflect its functioning when administered to the biological model. It was because of this that we

began to work with dimethyl, diethyl, diisopropyl and p-butyl derivatives, which we had wide experience with as well as plenty of bibiography. The selection of the biological model was of fundamental importance since we thought it should reflect the hepatic passage and the bladder uptake at the same time it would allow us to extrapolate from these results for use in humans. The use of mice allowed us to infer both passages. Since there is no information whatsoever on the use of this model in evaluating chelates with hepatobiliary affinity, we began by determining the value of the LD-50 for the first four derivatives in order to compare them with the new compound and thus be able to calculate the mass needed to reach a safety factor acceptable to pharmacopoeia and guarantee its future use in humans.

The use of the carotidea-carotineana bypass allowed us to estimate the degree of renal excretion in the radio-pharmaceutical and the speed of hepatic uptake. Although the latter showed no significant differences among the different N-derivatives, the renal excretion did. This leads us to affirm that the variation in molecular weight and lipophilic power bring about a variation in the movement and degree of metabolization in the hepatocyte which is reflected, precisely in different renal elimination.

If we consider that the T½ long was 92 minutes for the dimethyl derivative, 116 minutes for the diethyl derivative and 130 minutes for the diisopropyl derivative, we can relate the importance of the increase in molecular weight in renal excretion, and when we analyze that for the p-butyl derivative, this value is 238 minutes,we see the importance of the position of the substitute in the ring.

By introducing a variation in the hydrocarbonate chain, we maintained the type and position of the phenylic substitute constant. In this way, we obtained a T½ of 136 minutes which, compared to that obtained for the diisopropyl derivative, showed no significant differences.

When we compared the results of the lipophilic power,

we found that the dimethyl derivative showed -2.43±0.2; the diethyl derivative -0.70±0.2; the diisopropyl derivative 1.69±0.19 and the p-butyl 2.79±0.1 whereas the new derivative showed 1.90±0.19. Note that the lowest degree of lipophilicity appears in dimethyl derivative which, consequently, shows the fastest flow through the hepatobiliary structures and, as was proved by reinjection techniques, the lowest rate of metabolization. When analyzing the results obtained with the reinjections of the various radiopharmaceuticals, it was seen that when administering urine, renal elimination of radioac tivity was rapid, which shows an important decrease in the degree of lipophilicity and accordingly, an increase in hydrosolubility. The reinjection of the biliary contents produced a bladder uptake equal to the normal product and lesser increase in renal excretion than that detected for urine.

The parameter we decided to extrapolate from the biological model for use in human beings was the radiopharmaceuticals' transit times for the different structures. The results tell us that the maximum uptake time in the gallbladder was 15 minutes for diisopropyl derivative and for the new derivative. When these are compared with those obtained in normal human volunteers, we see that there are no significant differences since the diisopropyl derivative shows a VAT of 12.9±2.7 minutes and the new derivative 13.7±4.7 minutes.

This demostrates the validity of our biological model, allowing us to affirm that the mouse is the most adequate animal for experimenting radiopharmacology of hepatobiliary agents.

As for the new derivative, we found that it is potentially useful in the diagnosis of hepatobiliary diseases, showing rapid bloodstream transit and good hepatic uptake, a T½ of 2.0 minutes, high biliary accumulation, 8.8±3.6 percent of the dose administered in 15 minutes, and low renal excretion.

Finally, the reason for such biological behavior of the

diisopropyl and the new derivative should be explained. The results from the reinjection techniques of urine and gall-bladder show that the modification introduced in the molecular weight did not produce changes in biological behavior since it did not lead to modifications in lipophilic power, 1.69±0.19 for diisopropyl and 1.90±0.19 for the new derivative, and this parameter is really the one that regulates cellular flow of these radiopharmaceuticals.

ACKNOWLEDGEMENTS

We would like to thank the nuclear medicine branches that tested these radiopharmaceuticals enabling us to realize the extrapolation from our experimental results.

REFERENCES

Synthesis and radiolabeling of technetium radiopharmaceuticals based on N-substituted iminodiacetic acid:effect of radio-labeling conditions on radiochemical purity.
Fields.A.T, Porter.D.W, Harvey.E.B and Loberg M.D
J.Lab.Comps.Radiopharm. 15 (387) 1978

A mathematical model for the liver uptake and excretion of diethyl-IDA.
Gelius.L, Skretting.A and Aas.M. Eur.J.Nuc.Med.6 (139) 1981

Structure-activity relationships for Tc-99m benzimidazolyl methylimino-diacetic acid hepatobiliary radiopharmaceuticals.
J.Lab.Com.Rdaiopharm. 18 Nro 1 (191) 1981

Correlation analysis of substituents efects on the pharmaco kinetics of hepatobiliary agents.
Jansholt.A.L, Scheibe.P.O, Vera.D.R, Kron.K.A and Stadalnik.R.C
J.Lab.Comp.Rdaiopharm. 18 (198) 1981

Radiopharmaceuticals for hepatobiliary imaging.
Chervu.L.R, Nunn.A.D and Loberg.M.D. Sem.Nucl.Med. 7 Nro 1 1982

Comparative in vivo kinetics of some enw Tc-99m labelled acetanilido iminodiacetates.
Chiotellis.E, Varvarigou.A and Koutoulidis.C
Eur.J.Nucl.Med. 6 (241) 1981