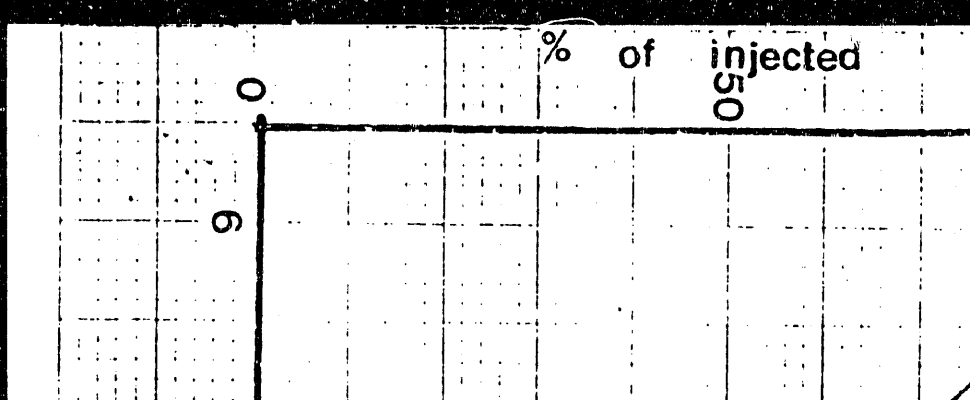
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NOTICE

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CONTRACT NUMBER DE-AC04-81EV10596

DEVELOPMENT OF BROMINE-77 FROM THE LAMPF FACILITY

FRED A. METTLER, JR., M.D., M.P.H., PRINCIPAL INVESTIGATOR

DOE/EV/10596--T2

DE91 000113

OBJECTIVE

The objective of the work is to conduct the necessary studies required to evaluate the efficacy, potential benefit and role of Bromine-77 labelled steroids in the detection and evaluation of treatment for hormone-dependent tumors.

TASKS TO BE PERFORMED BY THE CONTRACTOR

1) An initial investigation will concentrate on the radiobromination at Carbon 6 in selected simple steroids utilizing the nuclides of Bromine-82 nad Bromine-77.

2) Conduct analytical spectroscopy of radiolabelled compounds.

3) Investigate the biodistribution, toxicology and tumor affinity of labelled agents.

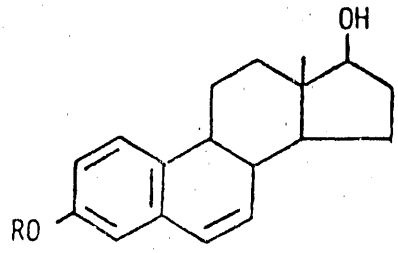
PROGRESS

The progress for tasks (above) 1 and 2 are outlined as follows.

This work has been performed by Dr. Mark Hylarides who is the synthetic biochemist hired under the terms of the Contract. His work has been conducted predominantly at the University of New Mexico, although there have been cooperative efforts with Dr. Scott Wilbur at the Los Alamos Radiochemistry Division (CNC-3).

Organic Synthesis:

The synthetic goals of the project are to prepare estradiol derivatives which are labeled with ^{77}Br at specific positions in the steroid nucleus. Substitution in the B and C rings is favorable since binding affinities of the resultant adducts will be similar to that of unsubstituted estradiol. In order to introduce ^{77}Br at these positions "protected" $\Delta^{6,7}$ estradiol 1, $\Delta^{7,8}$ estradiol 2, and $\Delta^{9,11}$ estradiol 3 are the necessary precursors.



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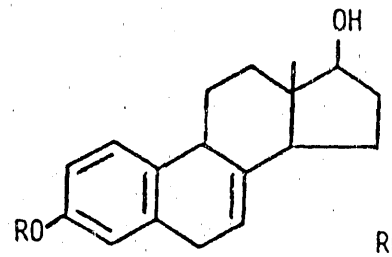

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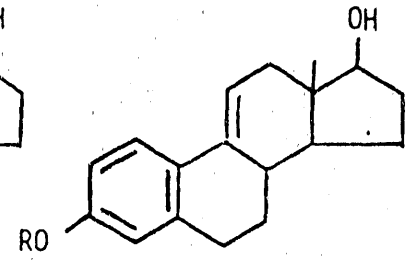

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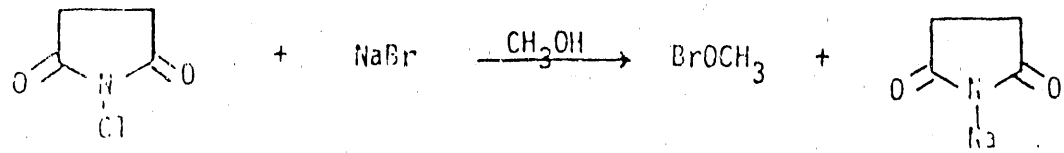
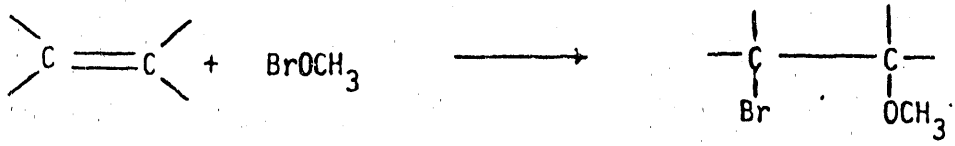
In order to eliminate the possibility of labeling in the A ring the phenolic hydrogen has to be protected, preferably with an acetate or benzoate.

The key step for the introduction fo the ^{77}Br label would involve addition across the double bond with an electrophilic bromide (Br^+). Br^+ can be prepared *in situ* by a variety of methods, including the formation of $BrOCH_3$. This reagent is easily prepared by the reaction of N-chlorosuccinimide with NaBr in methanol.

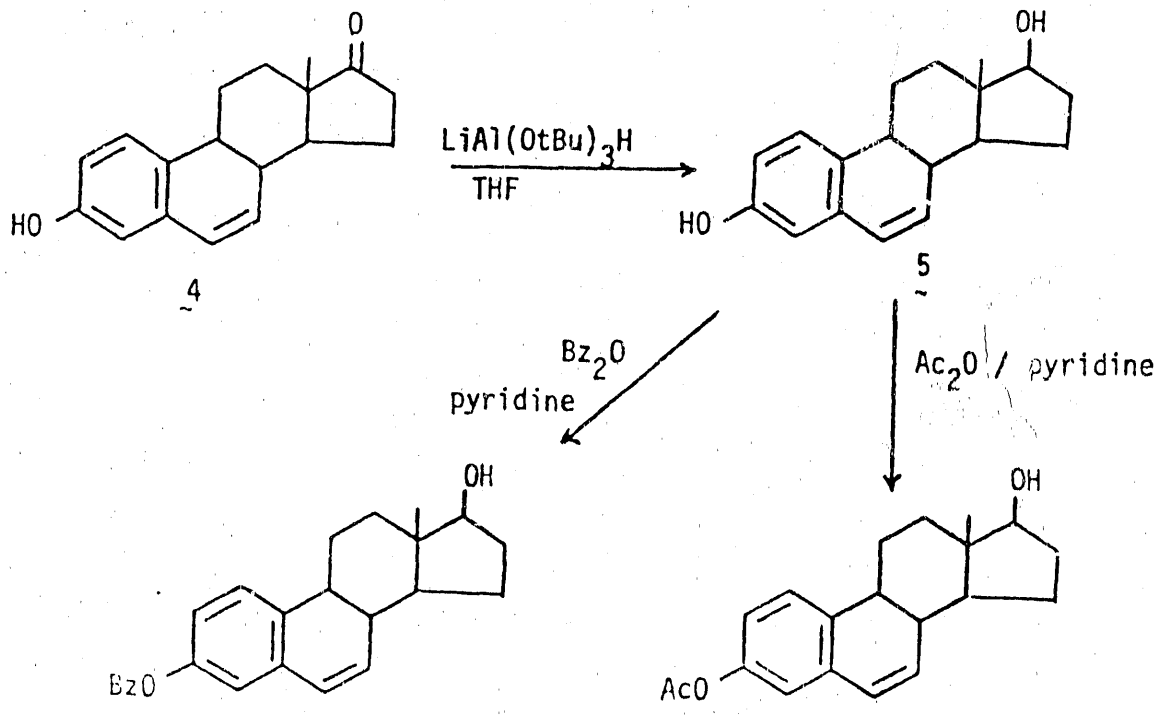


The highly reactive $BrOCH_3$ readily adds across double bonds to form bromo-methoxy adducts:



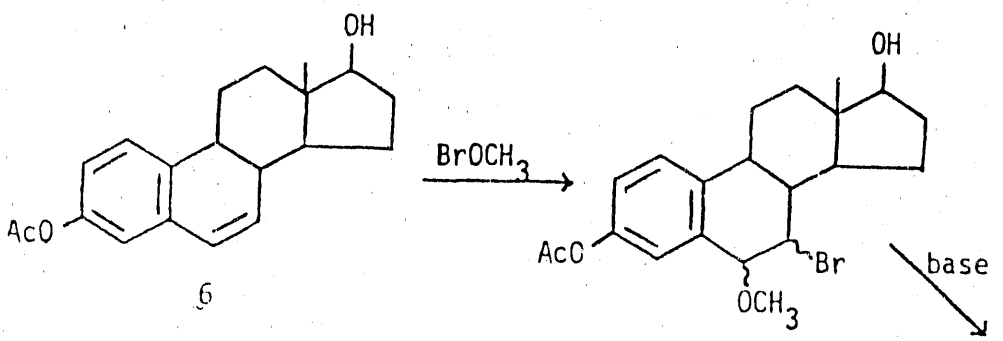
The regioselectivity of the Br^+ can be determined by the relative stability of the intermediate carbocation that is formed. Therefore, the reaction of 1 with $BrOCH_3$ should generate predominately the 7-bromo adduct. 2 would be used to prepare the 8-bromo adduct and 3 the 11-bromo adduct.

The initial problem involved the synthesis of the desired precursors. $\Delta^{6,7}$-estradiol-3-benzoate (and acetate) 6 was prepared from the readily available 6-dehydroestrone 4. Selective reduction of 4 with lithium tri-tert-butoxyaluminum hydride gave 6-dehydroestradiol 5 in high chemical and stereochemical yield. The phenol was then protected as the benzoate 6 and later the acetate. The acetate proved more beneficial as a protecting group due to its ease of removal with weak base.



4 5 6

The reaction of "cold" $BrOCH_3$ with $\Delta^{6,7}$estradiol-3-acetate 6 showed rapid incorporation of bromine into the steroid nucleus. Product formation was easily followed by high performance liquid chromatography. Subsequent addition of base (Na_2CO_3) to the mixture showed dissappearance of the initial product peaks followed by formation of new more polar adducts:



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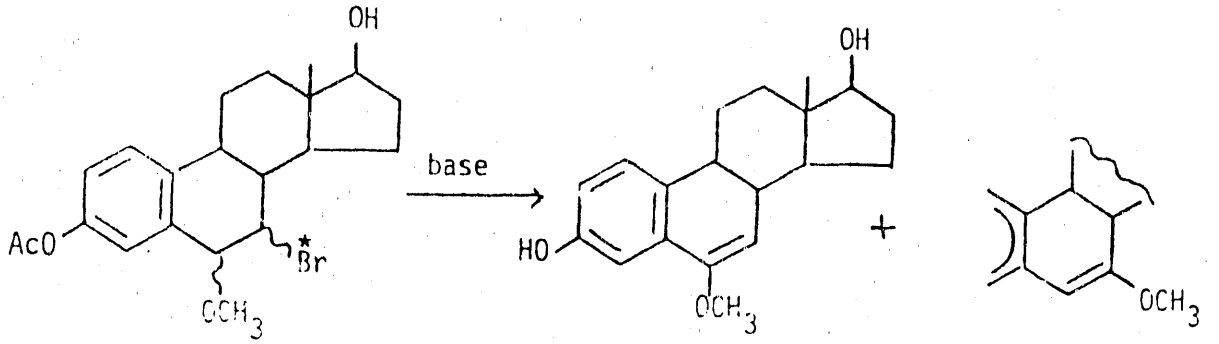
Polar products

The reaction of carrier-free ^{77}Br with $\Delta^{6,7}$estradiol-3-acetate 6 showed definite radioincorporation into the steroid nucleus. Attempts to remove the acetate group with base resulted in loss of all activity. We propose that deprotection was accompanied by dehydrohalogenation (-HBr) which generated the methoxy adducts 7.



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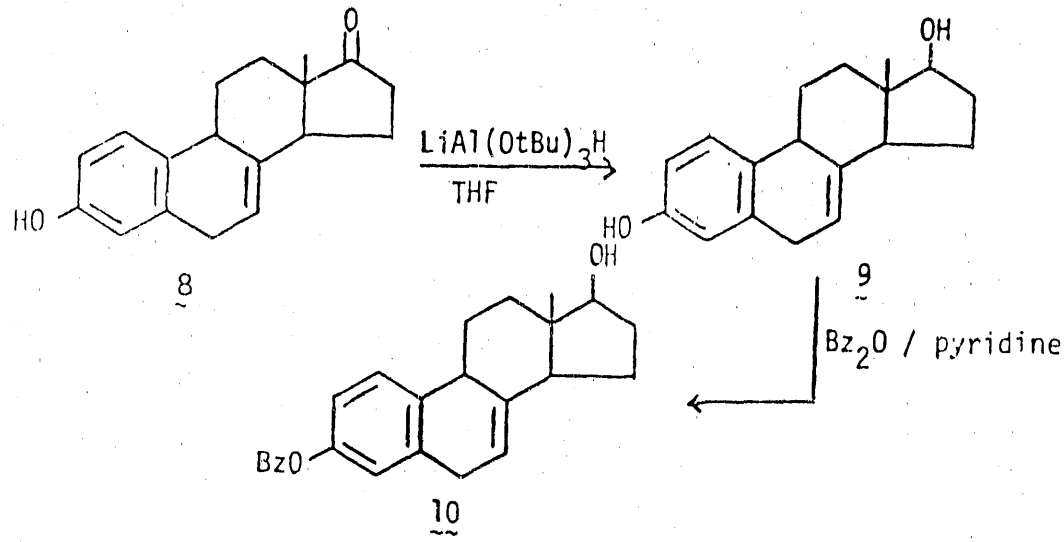
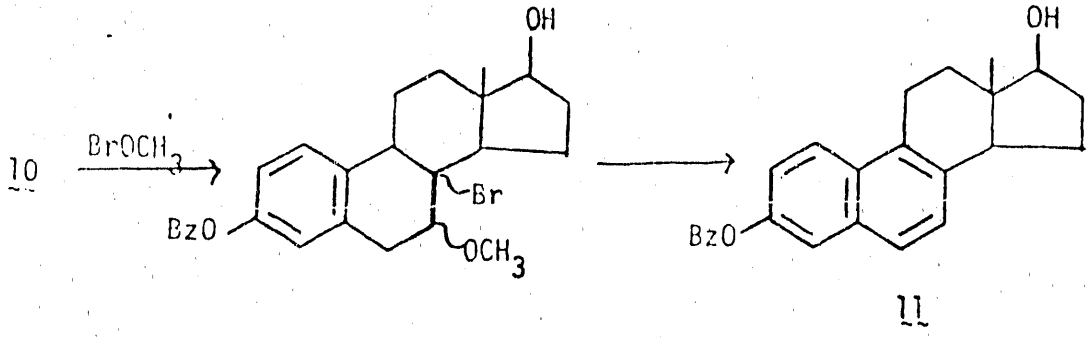
It was concluded that this position was too labile due to the acidity of the benzylic hydrogen. It is also probable that a similar dehydrohalogenation would occur in biological systems thus rendering the adduct useless for binding affinity studies. Further bromine incorporation studies were performed on the $\Delta^{7,8}$ and $\Delta^{9,11}$ estradiol-3-acetates (and benzoates).

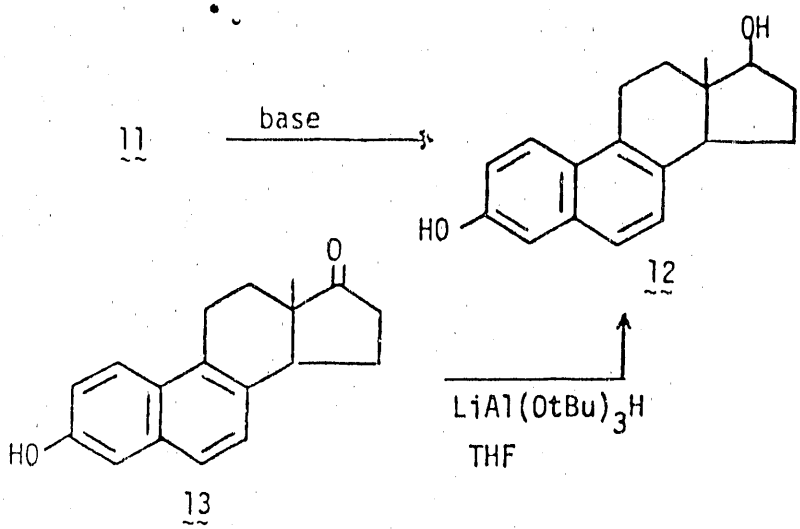
17β-Dihydroequilen-3-benzoate 10 was prepared by initial reduction of equilin 8 with lithium tri-tert-butoxyaluminum hydride to form 17β-dihydroequilen 9. The 3-benzoate 10 was subsequently prepared by the reaction of 9 with benzoic anhydride in pyridine.



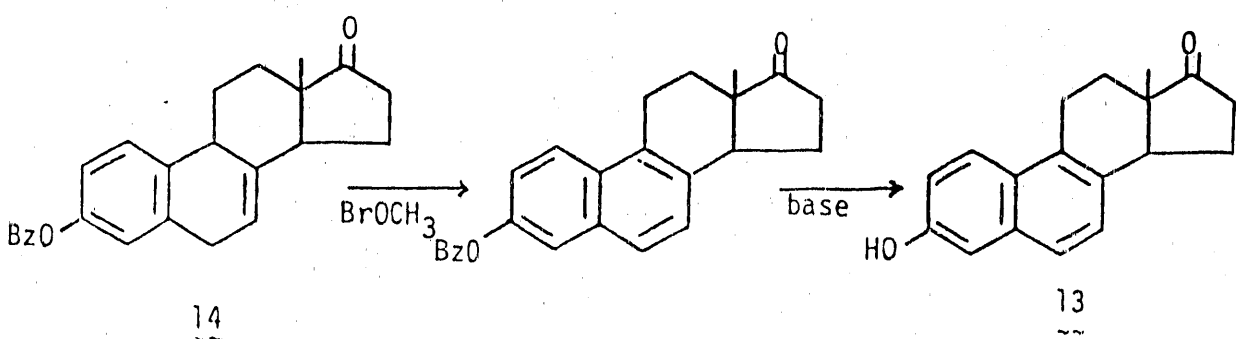
The reaction of "cold" $BrOCH_3$ with 10 showed rapid and quantitative formation of a single product; however, the analogous reaction with ^{77}Br showed no incorporation of ^{77}Br into the steroid nucleus. The reaction product was isolated and analyzed by ^{1}HNMR, ^{13}CNMR and MS. The data demonstrated that both the A and B rings of the steroid nucleus were aromatic. It was concluded that the addition of $BrOCH_3$ was followed by a rapid double elimination (-HBr and -CH_3OH) forming the stable equilenin system 11.



The product 11 was confirmed to be 17β-dihydroequilenin-3-benzoate since removal of the benzoate with base produced a product identical to the product obtained from the reduction of equilenin 13, i.e. 17β-dihydroequilenin 12.

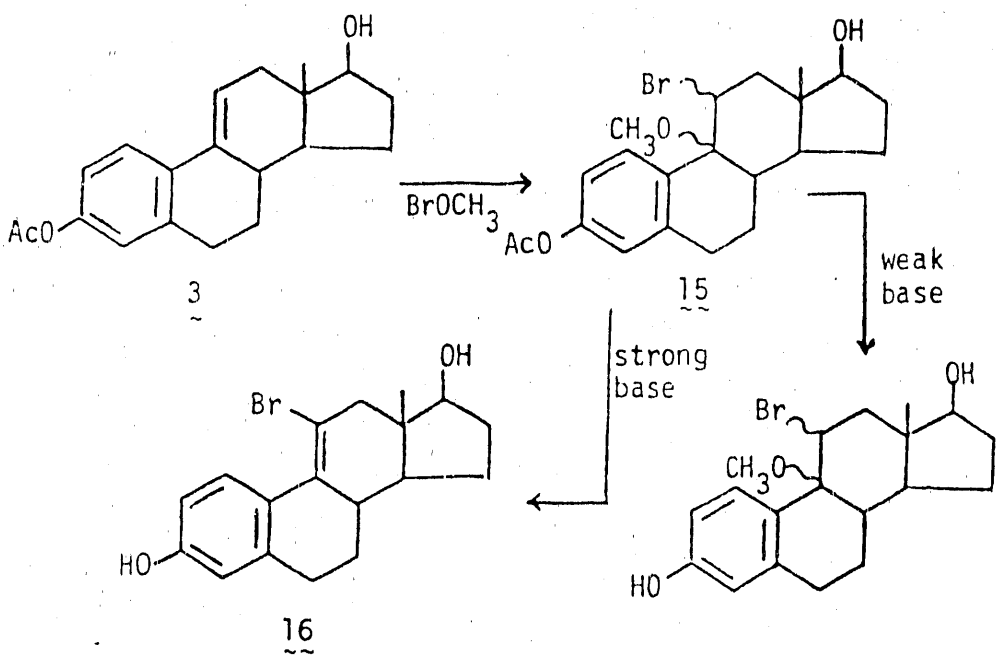


The reaction sequence was also performed on synthetically available equilen-3-benzoate 14. Reaction of 14 with $BrOCH_3$ followed by removal of the protecting group gave a product identical to equilenin 13 (^{1}HNMR, ^{13}CNMR and MS).

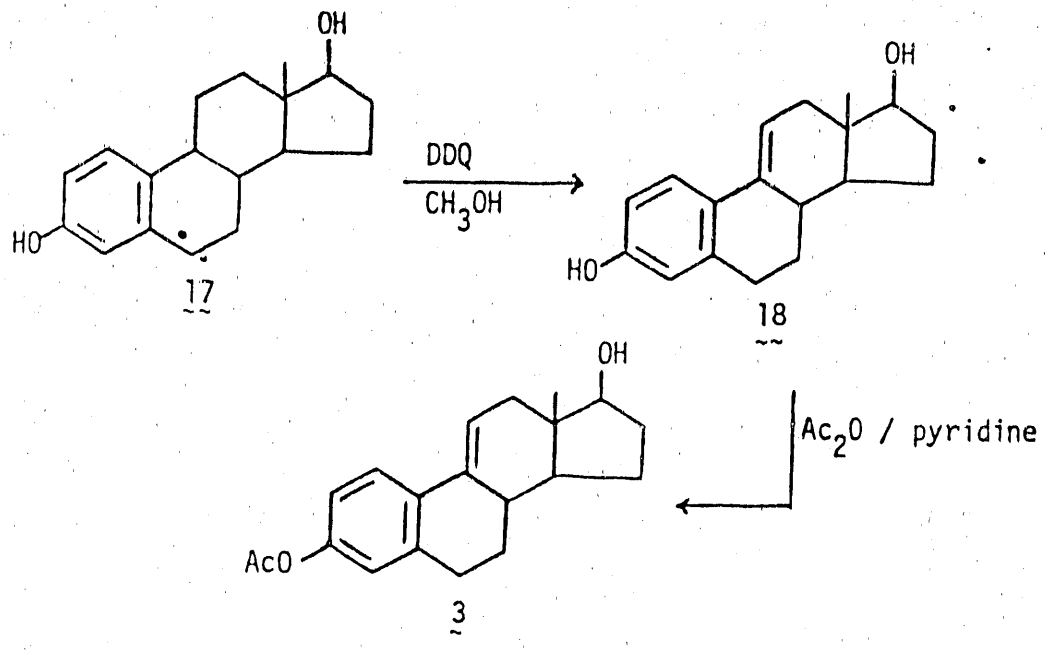


This reaction is of definite synthetic interest; however, is not applicable for radiobromination.

Present studies are concentrated on the reaction of $BrOCH_3$ with $\Delta^{9,11}$estradiol-3-acetate 3. This precursor appears to be the most promising since the corresponding product(s) would not contain an acidic benzylic hydrogen. Consequently, dehydrohalogenation or double elimination is not favorable under mild basic conditions. The predominate product is expected to be the 11-bromo adduct 15. Elimination under severe conditions would result in loss of CH_3OH thus generating the very stable vinyl bromide 16. This bromo-substituted estradiol may be very effective in binding studies.

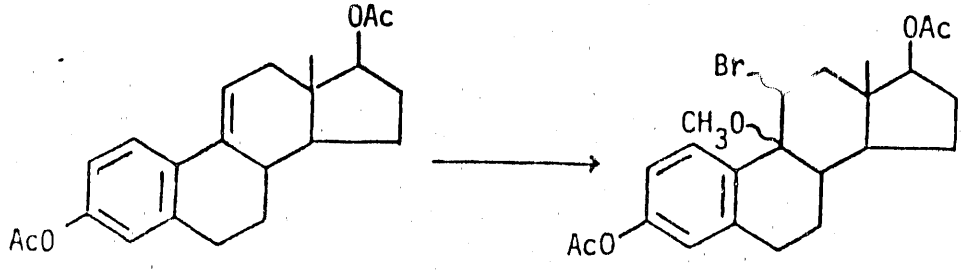


Work is presently underway to prepare pure $\Delta^{9,11}$estradiol-3-acetate 3. The synthetic scheme involves the reaction of estradiol 17 with DDQ. ^{1}HNMR of the crude product shows a 90% conversion to $\Delta^{9,11}$estradiol 18. Separation of the product from starting material is a difficult task, however, preparative HPLC will be utilized during the week of January 4-8 to obtain pure material. Further acetylation of 18 by standard methods will produce the desired precursor 3.



17 18 3

The reaction of ^{77}Br with $\Delta^{9,11}$estradiol-3,17-diacetate 19 has been investigated by our group. Results show a rapid incorporation of the ^{77}Br into the steroid nucleus. The subsequent addition of base did not result in loss of radioactivity. The 3-acetate was removed; however, more severe basic conditions are necessary to remove the 17-acetate.



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Preliminary results suggest that the $\Delta^{9,11}$estradiol-3-acetate 3 will be very effective for the incorporation of ^{77}Br. After isolation and characterization of the adduct studies will be underway to determine its binding affinity towards receptor sites.

ANIMAL STUDY

At the present time animals are being maintained in the Animal Research Facility at the University of New Mexico. These are rats carrying the following tumors lines: H-35 hepatoma and RFT sarcoma (both obtained through the courtesy of Dr. Hubner, Oak Ridge National Laboratories), 3M2N and 13762E mammary adenocarcinomas. These latter two are DMBA induced tumors, both of which are felt to be estrogen sensitive. These tumors have been maintained and transplanted with excellent success for over five months at the University of New Mexico.

Within the last three weeks biodistribution studies with tritiated estradiol and an estrogen sensitive mammary adenocarcinoma have been conducted. The data have not been analyzed yet but this should be done within the next several weeks.

The purpose of this was to assure that our techniques for biodistribution studies were adequate and to serve as a control basis for the synthetic studies. These animals and tumor lines will continue to be maintained in order to be ready for Bromine-77 labelled compounds.

IMAGING STUDIES

It has become apparent that imaging of Bromine-77 with the commercially available all-purpose collimators is going to be extremely difficult. Under the direction of Dr. James Christie and Dr. Charles Kelsey, work has begun to establish the modulation transfer function and observer performance of contrast and spatial resolution with differing collimators. This work has only been under way for several weeks, but clearly will be very important if Bromine-77 itself is to be utilized as an imaging agent in labelled compounds. To the best of our knowledge this particular point has not been addressed by any other investigators. The second year of the study is expected to focus on the collimator more closely and perhaps include development of a specific and inexpensive collimator for Bromine-77.

COOPERATIVE STUDIES

After discussion with Dr. Joop Thiessen, two additional areas of cooperative effort have been established by myself and Dr. Christie. It was felt that both of the projects would

be of benefit to the Department of Energy and would not require additional funding in order to be performed. The first of these projects is: Biodistribution and Tumor Imaging with Copper-67 citrate.

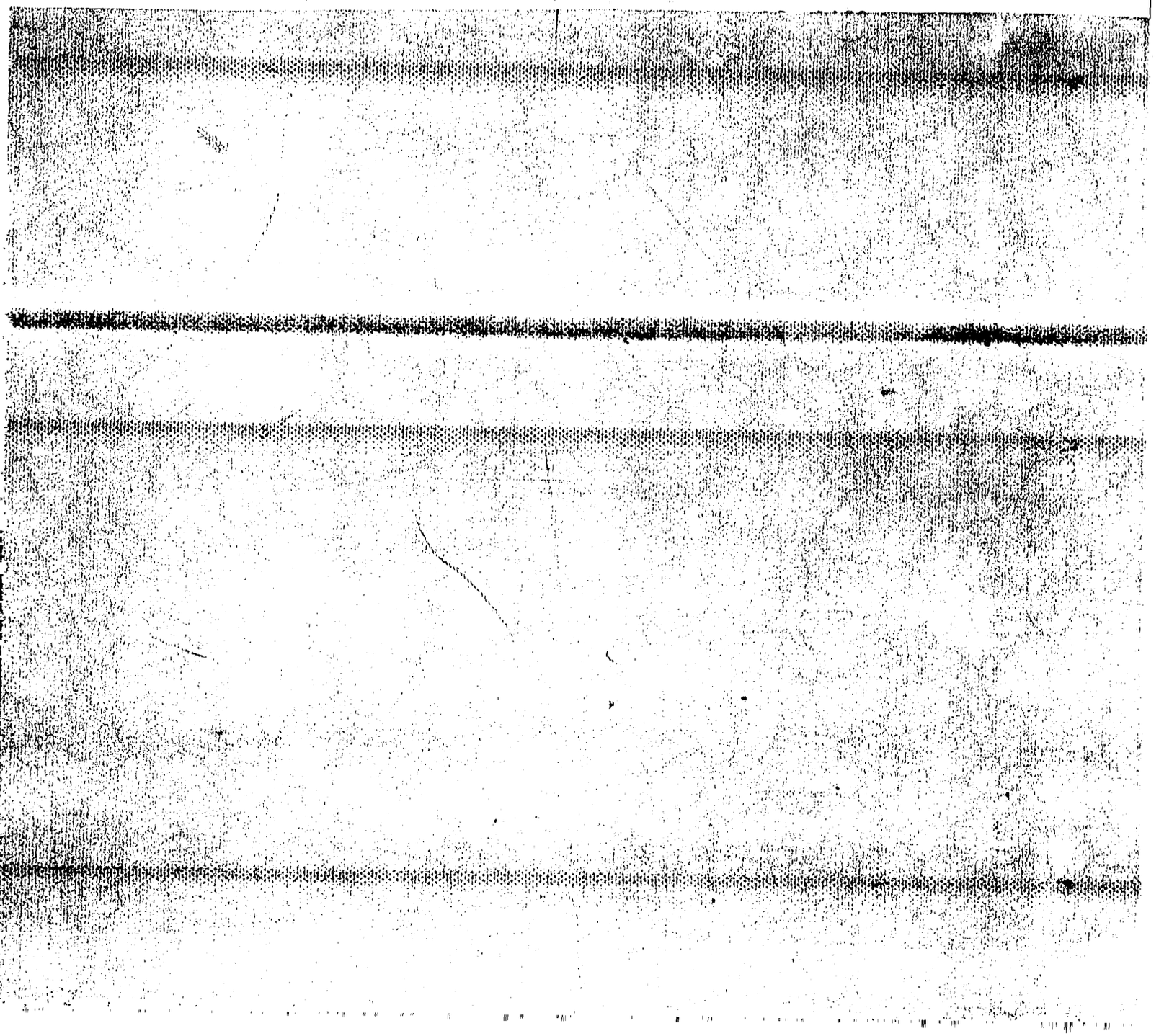
Three shipments of Copper-67 citrate were obtained from Dr. O'Brien from Los Alamos. Three separate biodistribution studies were performed in Fischer 344 rats. These included studies done examining the effect of carrier Copper, as well as the effect of intraperitoneal and intravenous injections. The results of these studies are indicated in the attached graphs. The biological half-life of Copper-67 appears to be approximately 36 hours with the effective half-life being 24 hours. There is little difference in distribution by route of injection and no significant difference was obtained with a moderately large amount of carrier Copper present. Biodistribution studies compare favorably with those published in the literature for Copper-64.

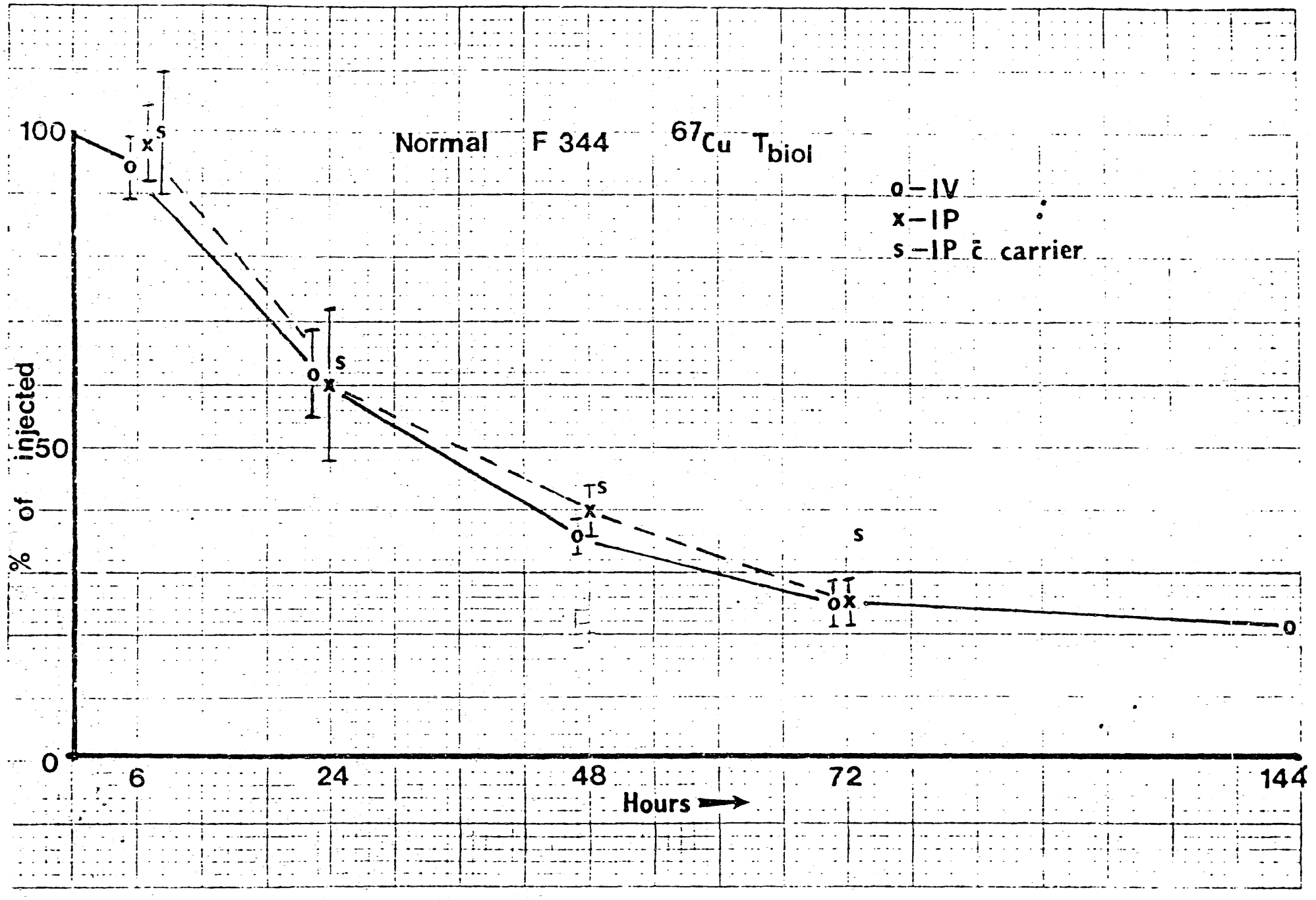
The possible value of Copper-67 citrate as a tumor imaging agent was evaluated with the H-35 hepatoma and the RFT sarcoma. A comparison that was made with Gallium-67 citrate and these tumors were chosen because of their known affinity for Gallium and since they were used originally in the development of Gallium by the Oak Ridge Group. Additionally, the mammary adenocarcinomas were also utilized. The first two experiments indicated tumor affinity for Copper-67 citrate. The best images were obtained at approximately 48 to 72 hours and compared very favorably with Gallium-67 citrate. In fact, by utilizing the various photopeaks of Copper, the tumor imaging obtained was subjectively as good as those obtained with Gallium.

Since Copper-67 citrate appears to have approximately the same tumor affinity as Gallium-67 citrate, an attempt was made to examine abscess affinity. Subcutaneous and intramuscular abscesses were developed utilizing Staph. Aureus and liver homogenate. While the abscesses were clearly imaged with Gallium-67 citrate, there appeared to be no affinity with the Copper-67 citrate. Thus Copper-67 when utilized as a citrate appears to be more tumor specific than Gallium-67 citrate. This work will be submitted for publication within the next several months and this Contract will be credited.

Examination of the modulation transfer functions and collimator performance on gamma cameras is also being currently examined as with the Bromine-77.

2) Under cooperative projects, in order to cooperate fully with other DOE funded facilities, Dr. Christie and the Division of Nuclear Medicine at this institution have agreed to participate in the evaluation of Ytterbium-169 citrate. This project is sponsored by Dr. Karl Hubner from Oak Ridge National Laboratories and we expect to be a co-investigator on this project with the isotope in preparation occurring at Oak Ridge. This particular cooperative project is expected to have no financial impact on the current Contract.

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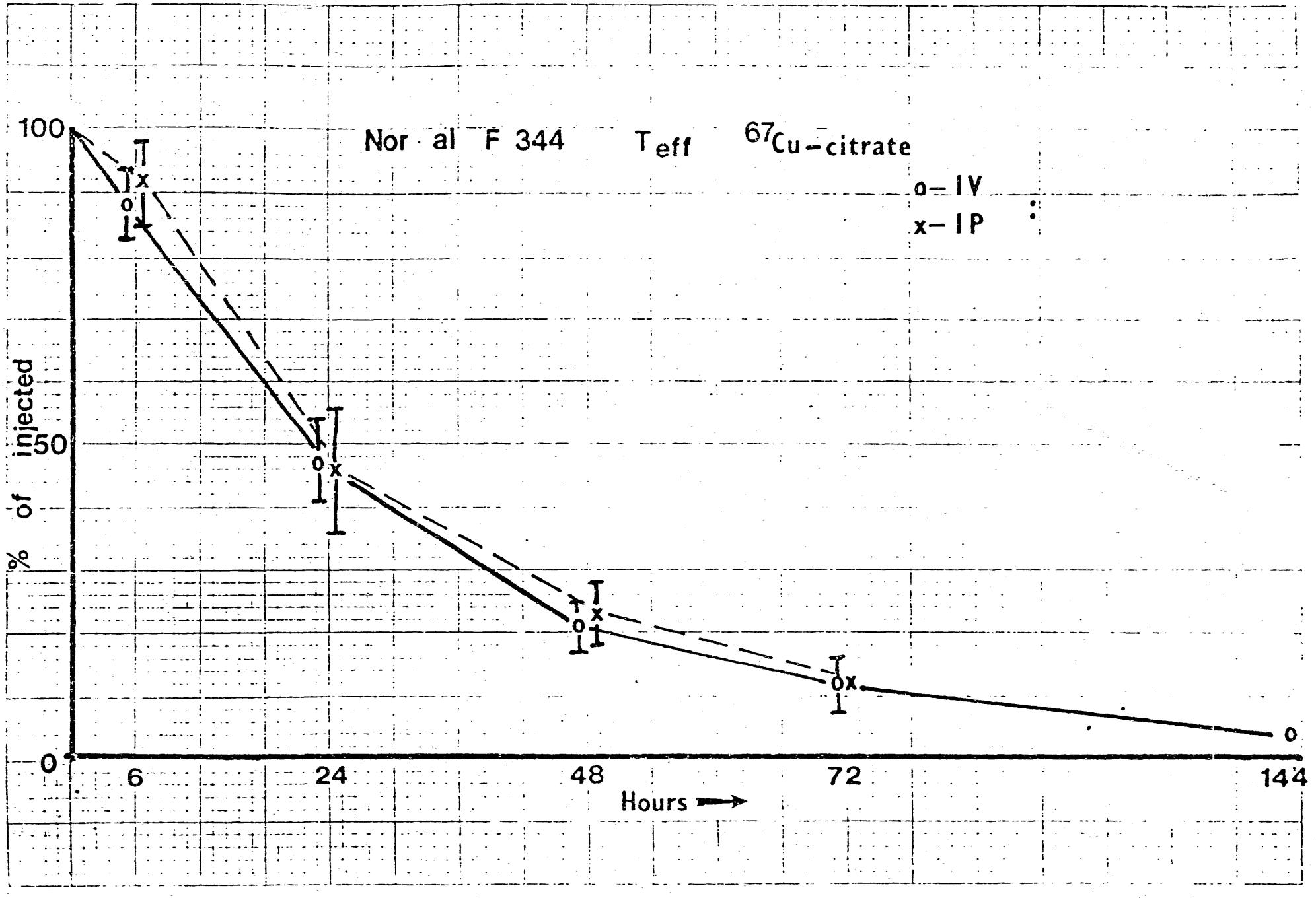
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