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Seattle 4, Washington

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Title

EFFECTS OF IONIZING RADIATION ON THE TESTICULAR FUNCTION OF MAN

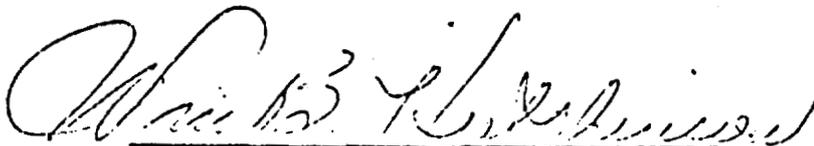
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	Fifth Year	"



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FOREWORD

The Division of Reproductive Physiology, under the direction of Dr. Carl G. Heller of the Pacific Northwest Research Foundation is uniquely able to undertake a program of the type proposed: Effects of Ionizing Radiation on the Testicular Function of Man.

Dr. Heller has spent his entire research career in the study of both male and female reproductive physiology with over 75 publications in this field. He and his co-workers devised the first successful clinical methods of assaying urinary gonadotropins and later urinary estrogens. His work, at the theoretical level as well as at the clinical level, has contributed to the understanding and interpretation of the female climacteric and also the less well understood male climacteric. He has been concerned with the characterization of new hypogonadal syndromes, the evaluation of hormonal variations in the female menstrual cycle, and the more precise definition of abnormalities found in Turner's syndrome and other forms of female hypogonadism. This has led to the investigation of the more delicate hormonal alterations in men and women which resulted in elucidation of causes of infertility and the effects of anti-fertility agents. Recently Dr. Heller, together with Dr. Yves Clermont of McGill, clarified the details of the histology of the human testes -- the number of stages in spermatogenesis, the cycles, and the overall rate of spermatogenesis. Thus, he and his staff, including two consultants in radiation, are particularly well qualified to evaluate the subtle effects that may be produced by exposing the human testis to radiation.

Since 1956 Dr. Heller has had a working arrangement with the Oregon State Penitentiary in Salem to conduct research on inmate volunteers. With their cooperation, he has conducted a wide variety of research on the physiology of reproduction of normal men and women. Visits to the penitentiary are made on the average of once weekly; and, as one prisoner calculated recently, Dr. Heller has spent enough time behind bars to have completed a one-year sentence (with time off for good behavior!). Considered neither a "cop" nor a "con", he has the kind of rapport so necessary for investigations with a convict population.

The Pacific Northwest Research Foundation provides a home for the Heller research team. It was incorporated as a tax-exempt organization in 1956 "for the express purpose of providing space and facilities for medical research by the physician or surgeon with an inquiring mind". Each division of the Pacific Northwest Research Foundation is essentially autonomous, although guidance for the Foundation's various functions is provided by three standing committees: the Committee of Investigators who exchange scientific ideas and manage housekeeping; the Medical Board who help decide policy and approve research projects; and the Board of Trustees, a lay group, who provide community support. Through the generous assistance of Swedish Hospital, the Pacific Northwest Research Foundation and now has a 10-year rent-free lease of the two top floors of Eklind Hall, formerly used as a nurses' dormitory, and the permission to construct five new stories above the present Eklind Hall auditorium. For the remodeling and construction of these facilities which are now

underway, the U.S. Public Health Service has granted \$280,000 with the Pacific Northwest Research Foundation matching these funds from private contributions. The new laboratories of the Reproductive Physiology Division will be completed within a few weeks.

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EFFECTS OF IONIZING RADIATION ON THE TESTICULAR FUNCTION OF MAN

February 1963

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EFFECTS OF IONIZING RADIATION ON THE TESTICULAR FUNCTION OF MAN

I. OBJECTIVES

We propose to apply known amounts of ionizing radiation directly to the testes of normal men in order to ascertain specific cytological and hormonal information. With respect to the cytological information, we propose: (1) to determine the exact nature of the cytological defect produced in the development of the germinal epithelium and to relate the extent of the defect to dosage and time; (2) to find the minimal dosage (and thereby determine dosage tolerance) that will affect the germinal epithelium; (3) to determine the time of recovery from any given dosage; (4) to determine the minimal dosage that leads to permanent damage of spermatogenic cells; (5) to determine the simultaneous effects of any dosage upon Leydig-cell cytology. With respect to hormonal information, we propose to determine the influence of any given radiation-produced testicular alteration upon other parameters such as: (1) total gonadotropin and interstitial-cell hormone (ICSH) excretion, (2) estrogen excretion, and (3) androgenic hormone excretion.

II. APPROACH

Ionizing radiation will be applied directly to the testes of inmate volunteers of the Oregon State Penitentiary. In preliminary experiments it is planned to determine range of dosage and the nature of the defect by irradiating only one testis, one-half testis, or a marked area of one testis (a circular area of 1 cm. diameter, for example). To aid in determining the radiation-induced interference in the progression of spermatogenesis, thymidine- H^3 will be injected at the beginning and/or at propitious times after radiation exposure. Testicular biopsy specimens will be obtained at appropriate times after the radiation exposure. If only a small area is irradiated, then only one post-radiation biopsy per individual per given series will be performed. If a greater area is exposed, serial biopsies on the same individual may be performed. The timing of the biopsies will be related to the timing of the cycle of the germinal epithelium -- at 16-day intervals and after an entire evolution of the spermatogenic process, 64 days -- to follow rate and denuding; and at 12, 24, 48, and 72 hours to follow degeneration, mitosis and meiosis.

After the nature of the cytological defect produced by irradiation has been determined and the range of dosage approximated, irradiation of all of both testes (the entire testicular mass of both testes) will be conducted. At this juncture the main portion of the investigation will begin. This will encompass measuring each of the peripheral effects (or parameters) of inducing a lesion in the human testis by

irradiation. Included will be a study of the cytology of the germinal epithelium and of the Leydig cells; the assessment of the urinary excretion of total gonadotropins and ICSH, and the determination of the estrogenic and androgenic excretion. The effect of radiation upon the ejaculate in terms of sperm numbers, motility, and morphology will be evaluated as well as the libido and sexual potentia of the subject. All of the above effects are routinely and currently measured in connection with the investigation of alteration of testicular environment due to drug administration. (1)

III. METHODS

A. HISTOLOGY

1. Cytology

Man is unique among commonly studied species such as rat, rabbit, guinea pig, ram, bull or stallion in being able to submit to testicular biopsy, and therefore to serial testicular biopsies, without the artifactual production of testicular damage or lesions (except perhaps in small area) by the act of performing the biopsy. We have demonstrated that by careful attention to the technics of obtaining the testicular biopsy*, each of the cells of the germinal series in man can be identified, placed in their correct stages (six for the human), and followed through each cycle (four for the human) to development of matured spermatozoa and their release into the lumena of the seminiferous tubules. (2,3,4)

With this armamentarium (and perhaps with the help of labelling of germinal cells with tritiated thymidine) we expect to be able to identify the cells of the human germinal series that are most sensitive to radiation; to determine at which stage in the development they are sensitive, and to follow the evolution of the cycles of the germinal epithelium until the damaged cells have disappeared. If a continuing inhibition to spermatogenesis is encountered as a result of irradiation, we hope to be able to explore the nature of this inhibition (again

* The immediate fixation in appropriately freshly prepared fixatives (Maximow's solution or Cleland's solution) and with appropriate staining technics (periodic acid - Schiff (PAS) and iron haematoxylin and eosin).

perhaps with the use of labelling by thymidine- H^3).

Quantitation of the morphological defect will be conducted using the subject's own pre-treatment biopsy as a control. We have recently worked out methods of cell counting using a Sertoli-cell correcting factor (and occasionally Abercrombie's formula) that reflects germinal cell alterations produced by hormonal or drug administration.

With the foregoing we should be able to detect whether radiation, for example, causes cytological abnormalities in the acrosomal system of the maturing or immature spermatids or whether it alters the development of leptotene, zygotene or pachytene primary spermatocytes, and also determine the effect upon degeneration of Type A, A₂ or B spermatogonia and pre-leptotene (resting) spermatocytes.

Once having established the nature of the cytological defect in one (or an area of one) testis, we shall have gained some knowledge of dosage and be prepared to irradiate both testes simultaneously in some subjects in order to observe other parameters.

Time is emphasized because it may be a key factor in understanding the effects of radiation on the testis. Oakberg (5) has found that radiation in mice produced denuding of all germinal elements except spermatogonia. It is known that radiation in man⁽⁶⁾ and other species⁽⁷⁾ will produce similar denuding effects. It is also known that hormones, hypophysectomy, and noxious agents may elicit the same end result in man. However, it is not known for man at which point in the development of the germinal epithelium the noxious agent acts,

whether it is agent-specific or whether all noxious agents have the same mechanism of action. Oakberg, in examining testicular histology in mice following irradiation, found that primary spermatocytes failed to be produced and that the more mature cells continued their normal development and separation from the Sertoli substance, not being replaced, until only rare spermatogonia remained. By studying this relationship between radiation effect and insult to spermatogonia he was able to determine the time of each cycle of the germinal epithelium for the mouse, as well as the total duration of spermatogenesis. By obtaining serial testicular biopsy specimens in man, we hope also to determine the nature of the defect in this species, as well as reconfirm the timing of spermatogenesis in man.

We plan, in general, to follow the experimental design of Oakberg^(5,9,10) who applied radiation to mice and so successfully made order out of chaos in interpreting the effects upon spermatogenesis. Since 1903 when Albers-Schönberg⁽¹¹⁾ described radiation effects upon testes, numerous observers have described the denuding process in many mammalian species. However, until clarified by Oakberg, it remained unresolved whether radiation caused cellular degeneration or affected mitosis. Two factors contributed largely to the resolution of the problem: (1) a careful identification of the cells, and (2) making the observations at the appropriate intervals following irradiation. With the aid of our collaborator, Dr. Yves Clermont, and the labelling with thymidine- H^3 , the identification of the cells for the human species has progressed enormously and should not prove to

be a barrier in our laboratory. With the awareness that spermatogonia of A and B types can undergo degeneration in hours or a few days following irradiation, and the awareness that cellular damage may not be revealed until a cell undergoes division, the appropriate intervals for obtaining testicular biopsies should be determinable. We are currently prepared to quantitate these changes (again following the approach of Oakberg). Currently such quantitation for normal control subjects and for those with alterations produced by administering hormones or drugs are being conducted daily in our laboratory by Mrs. Jean Fielding and Mr. George Needham.

We are prepared to follow the development of the surviving epithelium through the steps and rate of maturation to denuding. We are equally prepared to follow the steps and rate of the repopulation.

Unresolved is the question of which type of spermatogonia in the human is the more primitive cell -- type A₁(dark) or A₂(light). This problem may well be resolved by observing the surviving spermatogonia. Labelling with thymidine-H³ a few hours before and a few hours after irradiation may aid greatly to pinpointing the nature of the effects and may well elucidate such unresolved problems as where and when the first cycle of spermatogenesis begins in the human. Such a plan was used by Monesi in mice⁽¹²⁾.

We are also well aware that Roosen-Runge^(13,14) is unable to distinguish between the three types of spermatogonia and has not defined the resting or preleptotene primary spermatocyte.

2. Minimal Dosage

We expect to determine minimal dosage and, parenthetically, that dosage below which no harm is caused to the human germinal epithelium. In the present experiment we are speaking only of the effect of a single dose of radiation -- cumulative effects could be evaluated later. Having established the nature of the radiation effect upon human testes, the minimal effective dosage could be established by varying dosage. It is visualized that this could be established by combining the observations made before and after irradiation of (a) a single testis or its separate portions, or (b) both testes. However, the same result could be accomplished by either approach. In our investigation we wish to progress from (a) to (b) as rapidly as is prudent, so that we may soon be measuring more parameters of the effect of radiation upon testes in man.

3. Time of Recovery

The morphological recovery from radiation damage could be observed by examining repeated testicular biopsies. Equally important would be observing the recovery of each altered function following the irradiation of both testes. This therefore will be attempted as described under B, Hormone Assays.

Recovery from the insult of a noxious agent (presumably including radiation) may be prompt, delayed, complete, incomplete, or absent. To determine this for a given dose of X-irradiation will require serial

observations, in time, of morphology; evaluation of ejaculate; and hormone analyses.

4. Minimal Dosage Leading to Permanent Damage

Damage to testes may be incomplete, involving some seminiferous tubules permanently while others effect complete recovery of spermatogenesis. Thus the reserve capacity of the testes could be decreased while function remains normal; or it may be complete. The incomplete situation was observed by Pitcock in his report on "Late Testicular Lesions in Irradiated Monkeys"⁽¹⁵⁾. By following each of the parameters revealing testicular function, including serial testicular biopsy, the minimal dosage causing any permanent damage should be revealed.

5. Radiation Effect Upon Leydig-Cell Function

Irradiation of testes will expose equally the Leydig-cells, the Sertoli-cells, and the germinal epithelium. Although the latter cells are known to be most sensitive to radiation (which ones exactly for man, we hope to discover in this investigation), it remains unknown to what degree, if any, Leydig and Sertoli cells are affected. To the Leydig-cell has been ascribed the function of secreting testosterone and estrogen⁽¹⁶⁾, and to the Sertoli-cell the unlikely function of secreting "Inhibin" and/or estrogen. The latter claim was made as recently as September 1962 at the International Symposium on the Effects of Ionizing Radiation on the Reproductive System at Fort Collins, Colorado, by D. Lacy, who moreover claimed alterations in secretion with X-irradiation.

Therefore, for each radiation dosage, the cytology of the Leydig-cell and Sertoli-cell will be examined after staining with Masson's trichrome. Moreover, the excretion of estrogens and androgens will be determined. In order to perceive slight shifts in secretion, the estrogens will be measured in terms of their separate components - estradiol, estrone, and estriol; and testosterone along with the 17-ketosteroids in their separate components as etiocholanolone, dehydroisoandrosterone and androsterone. This will be done initially on urine samples, later, if feasible, on blood samples and, finally, if at all possible, on testicular biopsy specimens. At the same time shifts in the ratio of excretion of total gonadotropins to ICSH should reveal something of the functional integrity of the Leydig-cells. Clinical evaluation of libido and sexual potentia may afford a clue as well.

B. HORMONE ASSAYS

If, as we presume, irradiation partially denudes the germinal epithelium, and if pituitary gonadotropic hormones are utilized during the process of germinal cell maturation, then with declining germ cell proliferation less gonadotropins should be utilized and more be available to spill over into the urine. Therefore, total urinary gonadotropins will be assayed in each subject in whom both testes are irradiated. The expectation is that a sharp rise in urinary gonadotropins will be encountered. If Leydig-cells remain unaffected at lower doses of radiation, as they well might, no change in ICSH excretion is

expected. If Leydig-cell function is affected with higher doses, a rise in ICSH excretion may be encountered.

1. Gonadotropins

Gonadotropins will be concentrated using the speed-filter and kaolin adsorption method of Albert⁽¹⁷⁾. Total gonadotropin assays will be by the increase in rat ovarian weight method of Lauson, Heller, and Sevringhaus⁽¹⁸⁾. ICSH assays will be conducted using the male hypophysectomized rat method of McArthur⁽¹⁹⁾. Both total gonadotropin and ICSH assays will be expressed in units of a standard human gonadotropin preparation. Not yet distributed is Pergonal-23, which has been adopted by the World Health Organization as the International Reference Material. In the meantime, we have been using Pergonal-22c and, having exhausted our supply, will be using Pergonal-26. (Each is similarly prepared by the same manufacturer in Italy.)

2. Steroids

Currently this laboratory is using the bioassay method of Lauson, Heller, Golden, and Sevringhaus⁽²⁰⁾ for the estrogens. This is a nonspecific method chosen largely for its sensitivity (0.1 micrograms in terms of estradiol per 24 hours of urine) and its reliability. The current androgen assay method is also the nonspecific total 17-ketosteroid method of Dreker⁽²¹⁾.

The necessity of using newly developed, more refined analytical methods becomes apparent when it is considered that the proposed experiments may well be expected to define the actual sites of formation and the sites of interconversion in the human of the individual

estrogens (such as estrone, estradiol, estriol) and the individual androgens (such as testosterone, androsterone, epiandrosterone, and etiocholanolone).

The assay that we propose to adopt for the estrogens in plasma is essentially that described by Ichii⁽²²⁾. In outline this consists of the following steps. A quantity of plasma is treated with insignificant, but known, amounts of tritiated estrone and estradiol in order later to be able to calculate recoveries. It is then extracted with ether. The residue, after solvent removal, is dissolved in 70% methanol, cooled to -20°C overnight, centrifuged, and the supernatant partitioned against petroleum ether. The phenolic material of the alcohol fraction is then chromatographed in the Bush B₃ system. The zones containing the radioactivity are eluted with methanol and rechromatographed in the Bush A system and the B system for the estrone and estradiol, respectively. The purified eluted materials are then determined quantitatively using the phosphoric acid fluorescence method of Finkelstein⁽²³⁾ for the major portion of the sample while a small portion is counted in a scintillation counter to measure the over-all recovery. This method is sensitive to 0.002 micrograms in 50 ml. of plasma.

The assay that we propose to adopt for urinary estrogens is essentially a separation described by Gurside, et al⁽²⁴⁾ followed by quantification by the fluorescence method of Finkelstein⁽²³⁾. Briefly, this consists of the following steps: (1a) addition of tracer quantities of estrone, estradiol, and estriol; (1b) enzyme hydrolysis using

Glucosylase; (2) ether extraction and bicarbonate washing of the extract; (3) sodium hydroxide separation of the phenolic fraction; (4) neutralization to pH 8 of sodium hydroxide solution and re-extraction into ether; (5) evaporation of the ether from the extract and partition of the true solid between benzene and water to separate the estriol from the estrone and estradiol according to the method of Brown⁽²⁵⁾; (6) liquid partition chromatography of the two separate fractions using two phase systems (with the polar phase supported on Celite) composed of ethyl acetate, n-hexane, methanol, and water for the estrone-estradiol fraction; and carbon tetrachloride, n-hexane, methanol, and water for the estriol fraction; (7) quantitative determination of the separated estrogens by fluorescence and scintillation counting.

The method that we propose to adopt for plasma testosterone is essentially that of Finkelstein, et al⁽²⁶⁾, as modified by Forchielli⁽²⁷⁾. This method consists of the following basic steps. The sample is divided into two equal portions. To one portion is added C¹⁴ labelled testosterone of activity about 2500 c.p.m. and to the other is added H³ labelled estrone and estradiol of activity about 10,000 c.p.m. These samples are then treated in parallel by ether extraction, solvent evaporation, 70% methanol fractionation, removal of the methanol from the 70% solution, and extraction of the remaining aqueous solution with benzene followed by partition between the benzene and a 1 M sodium hydroxide solution. At this point the phenolic fractions in the sodium hydroxide from the parallel samples are combined (for estrogen determination if desired) while the neutral fractions are still kept

separate. These latter neutral fractions are separately dried and chromatographed in a ligroin-propylene glycol system of Savard⁽²⁸⁾ on paper and the testosterone region (as shown by C¹⁴ counts) eluted from both. The sample containing the C¹⁴ testosterone is incubated with a placental dehydrogenase which converts it to estradiol, while the sample originally treated with tritiated estrogens is taken up in ethyl alcohol (to inhibit the enzyme reaction), but otherwise treated in the same way. Both portions are reduced with sodium borohydride to convert any estrone to estradiol, extracted, rechromatographed in the Bush B₃ system, eluted, and determined quantitatively both by fluorescence and by counting. Recoveries determined by counting can then be combined with the difference between the two parallel samples to determine the blank correction due to the presence of estrogens in the testosterone sample. While this method seems complex, it has the advantage of specificity and sensitivity to 0.01 micrograms of testosterone per 50 ml. of plasma. It will furnish a standard for any gas chromatographic method that may be developed.

The method that we propose to adopt for urinary testosterone (and, perhaps, with suitable modifications eventually for plasma and tissue) is that of Futterweit, et al⁽²⁹⁾. This consists of enzymatic hydrolysis after addition of tritiated testosterone followed by ether extraction and removal of phenolic materials by washing with sodium hydroxide. The neutral fraction from the ether is treated with the Girard T reagent, partitioned between water and ether, the aqueous solution hydrolyzed, re-extracted into ether and evaporated to dryness. This

ketonic fraction is chromatographed twice by thin-layer chromatography first, using a benzene-ethyl acetate mobile phase; and then, using a cyclohexane-ethyl acetate mobile phase. The eluted testosterone is divided quantitatively into two portions and dried. One portion is taken up with ethyl acetate for scintillation counting, while the other portion is taken up in acetone for gas chromatographic determination on an SE-30 column at 200°C.

The method that we propose to use for the 17-ketosteroids is that described by Wotiz⁽³⁰⁾. This involves the hydrolysis, extraction, and separation of the neutral fraction of steroids similar to that described for urinary testosterone. The dried extract is treated under anhydrous conditions with hexamethyldisilazane and trimethylchlorosilane to form the trimethylsilyl ethers of androsterone, etiocholanolone and dehydroepiandrosterone as well as the trimethylsilyl ethers of 11-ketoetiocholanolone, 11-hydroxyandrosterone, pregnanediol and pregnanolone. These may be determined directly on an XE-60 column at 200°C with flame ionization detection. Alternatively, a "Hi-Eff 8B" liquid phase may be used at 240°C in place of the XE-60 in the known absence of pregnanediol.

All of the above methods are specific, sensitive, tested, and workable. Some of them are, however, more laborious than others. Thus, it is felt that some effort should be spent in concurrent development to effect the rapid and sensitive combination of prepurification of samples by thin-layer chromatography with quantification by gas

chromatography. This should, of course, be done with constant attention to the reliability of the faster methods in terms of their comparisons to the standard methods cited above and by monitoring against the bio-assay of the separated fractions.

It might be pointed out that the important problem in the development of a gas-chromatographic method is largely one of specificity. Once this has been assured, there is potentially the opportunity of a greatly increased sensitivity. This may be increased to the point of sensitivity to nanogram quantities (rather than microgram quantities) using electron capture detection of halogenated steroids.

IV. PREPARATION FOR THE PROJECT

Permission has been granted by C. T. Gladden, Warden, and Dr. Dan DiIaconi, Chief Medical Officer, of the Oregon State Penitentiary to conduct the investigation at the Penitentiary Hospital. Dr. E. Kenneth Vollmar, radiological consultant to the Penitentiary, has expressed interest in helping in any way possible. Dr. Vollmar proposed that the radiation be administered at Salem Memorial Hospital where he is Chief Radiologist and has the necessary equipment. However, the problem of providing the requisite guards and the hazard of attempted escape mitigated against this solution. The Warden agreed with the suggestion that we purchase a mobile or small x-ray therapy unit and donate it to the Penitentiary when the experiments are concluded.

Members of the Radiation Committee of the Oregon State Board of Health have been consulted, as was Dr. Thomas Dotter, Head of the Department of Radiology of the University of Oregon Medical School, and Mr. Bobbitt, legal consultant of the Oregon State Board of Medical Examiners, and the Secretary of the Oregon State Medical Society (of which Dr. Heller is a member) to determine whether any legal or ethical problems might be involved. The answer was unanimous that no barriers to the pursuit of the problem were foreseen. No special permission or dispensation seems required. A form for the signature of each volunteer (and wife, if any) will be worked out.

It was made clear to each of the above individuals consulted that we would be working with normal male convict volunteers who

either had been vasectomized or would be vasectomized before, during, or at the end of the series of experiments. The reason for vasectomy is to eliminate the possibility of contaminating the general population with radiation-altered chromosomes. During the pursuit of the investigation, the subject will, of course, have no opportunity to contaminate the population.

The inmate volunteers who have asked to join the program are chiefly motivated by the desire to have a vasectomy performed and the prospect of monetary gain. Each volunteer for this program (about 100 to date) is carefully screened by Dr. Heller. Consultation with the prison physicians and hospital attendants, as well as with their fellow convicts (the latter's advice often invaluable), helps determine the degree of reliability of each volunteer. No inmate whose religion does not condone vasectomy is considered. Usually younger convicts are not considered. Each candidate is then examined by the Penitentiary psychiatrist to attempt to determine the emotional consequences vasectomy may have upon him. Dr. Dan DiIaconi, and/or Dr. Heller perform the vasectomies. At the present time there are 38 screened subjects who have been judged to be reproductively normal and are ready to join the program. About 20 of these are already vasectomized, some having been on other programs.

The arrangements for the use of tritiated thymidine at the Oregon State Penitentiary have been made previously. The license of the Atomic Energy Commission (46-922-3) to the Tumor Institute of The Swedish Hospital of Seattle, Washington has been modified to

include H³. It has also been modified to include Dr. Heller's use of thymidine-H³ at the Oregon State Penitentiary at Salem.

In anticipation of the development of more refined and precise measurements of steroids in biological materials, Dr. Harry Christoffers attended the recent course in gas chromatography held at Boston University under the guidance of Dr. Herbert H. Wotiz, January 14-19, 1963 in Boston, Massachusetts.

V. PLAN OF ACCOMPLISHMENTS

We are prepared to proceed the moment the X-ray therapy equipment arrives and is calibrated. The Penitentiary subjects, the hospital, its physicians, two Oregon-licensed radiologists, the Pacific Northwest Research Foundation's histological laboratory, as well as the team's technical and professional personnel are all ready to conduct the investigation.

Two steps in the program will be initiated simultaneously: (1) the irradiation of portions of one testis in previously vasectomized subjects, and (2) the application of new analytical technics to bring the chemical determination of steroids to a greater degree of resolution on a routine basis.

Step 1. The irradiation for subsequent serial testicular biopsies would be performed using varying doses of radiation in a range, for example, of 50 to 200 roentgens in five subjects. Present for the irradiation would be Drs. Vollmar, Howieson, and Heller. Biopsies would be performed by Dr. Heller, and the histological work-up and analysis would be performed by Mrs. Jean Fielding and Mavis Rowley. Quantitative cytological studies would be made by Mr. George Needham. As soon as the nature of the cytological defect emerges and the correlation with dosage is made, another five subjects would be irradiated, perhaps this time incorporating thymidine- H^3 into the scheme for more accurate appraisal. As soon as a preliminary dosage schedule has been evolved with some degree of consistency, then both testes will be irradiated

and the other parameters measured, i.e., ejaculate, gonadotropins, estrogens, and androgens.

Step 2. Simultaneously with step (1) above, Dr. Harry Christoffers and assistant Ted Roscoe will pursue improvement of the steroid analysis methods. There may be some delay in acquiring all the necessary equipment. However, it is hoped that the new steroid methods will be developed to the point of application by the same time as the preliminary radiation-histology studies are completed.

Step 3. At this juncture we hope to combine the information of steps (1) and (2) to elucidate the subtle changes that might be produced when all of the germinal and hormonal testicular tissue in these subjects is irradiated.

Dr. Yves Clermont, Professor of Anatomy at McGill University, Montreal, has been an active collaborator in assessing the normal histology of the testes. He will continue in this capacity during the present investigation.

VI. FINANCIAL INTERACTIONS

A. OTHER RESEARCH SUPPORT

PHS GM-05961-05	Effect of Steroids Upon Spermatogenesis Grant Period, April 1, 1963 to March 31, 1964	\$38,640
PHS CA-04240-06	Effect of Progestins on Human Gonadotrophins Grant Period, April 1, 1963 to March 31, 1964	23,155
Wm. S. Merrill Co.	Effect of Clomiphene Upon Total Gonadotropins and ICSH Grant Period, January 1, 1963 to December 31, 1963	7,500
E. R. Squibb & Co.	Effect of Delalutin & Delatestryl upon Spermatogenesis (To be applied for)	4,000
No other applications pending		

B. INTERPLAY BETWEEN AEC PROPOSAL AND OTHER SUPPORT

The general design of our investigations into the area of male reproduction follows the outline proposed here, the major difference being that the agents used are hormones or drugs rather than radiation. The same parameters are measured; therefore, in general, the same personnel, equipment, and facilities are utilized for experiments supported by different agencies. For example, the individuals involved in histology prepare testicular tissue exposed to hormones as well as to radiation -- and the same microtome, microscopes, cameras, etc. can be utilized.

C. TRAVEL

Dr. Yves Clermont, our collaborator in Montreal, requests no funds other than for travel to Seattle and Salem to work with tissues obtained.

A reciprocal trip by two of our investigators to Montreal is also desired.

The current travel routine to Salem from Seattle (438 miles round trip) calls for transportation (air or car) for Dr. Carl Heller and an assistant every second week, and lodging for two nights in Salem, thus permitting two full days and two full evenings working at the Oregon State Penitentiary. Additional trips are required to perform testicular biopsies when the timing of spermatogenesis or interference of other penitentiary hospital activities dictates time intervals outside the regular schedule. Thus, 50 routine trips a year (25 per person) and 10 to 20 irregular trips are anticipated. At a minimum of \$50 per trip for 70 trips the cost will be \$3,500. Funds for these routine visits are already provided for by the U.S. Public Health Service. An additional \$1000 is being requested for trips occasioned by the more exacting timing requirements of this proposal.

Funds are desired for attendance at the Endocrine Society meetings, the Society for the Study of Fertility, American Society for Clinical Investigation, and/or the American Physiological Society, but are not being requested at this time. Funds have already been provided for attending the annual Laurentian Hormone Conference and the Western Society for Clinical Research.

D. STIPEND FOR INMATE VOLUNTEERS AT THE OREGON STATE PENITENTIARY

We are requesting \$200 per individual subject to pay for exposure to radiation, submission to several subsequent testicular biopsies, the collection of urine for hormone studies, the collection

of weekly seminal samples, and for vasectomies. The volunteers will be paid a token sum during the course of the investigation, and the remainder as a lump sum upon satisfactory completion of the study.

E. ALLOCATION OF PERSONNEL SALARIES

PRIVACY ACT MATERIAL REMOVED

Requested of AEC	Presently Available from PHS	Presently Available Other
------------------------	------------------------------------	---------------------------------

- | | |
|------------------------------------|----------------------------------|
| (1) Carl G. Heller, M.D., Ph.D. | Principal Investigator |
| (2) Harry J. Christoffers, Ph.D. | Senior Investigator |
| (3) Jean Fielding, A.B. | Senior Investigator |
| Ted Roscoe, B.A. | Research Assistant |
| Mavis Rowley, B.A. | Research Assistant |
| George Needham, M.S.
(1/2 time) | Research Assistant |
| (4) Stephen G. Heller, B.A. | Technician |
| (5) Gary V. Heller | Technician |
| Kearney Poyser | Secretary |
| John L. Howieson, M.D. | Consultant |
| E. Kenneth Vollman, M.D. | Consultant |
| Yves Clermont, Ph.D. | Consultant |
| To be named | Research Assistant
(steroids) |
| To be named | Technician |

(1) Proposes to devote 90% of his time to research, reserving 10% for referral consultation private practice in endocrinology.

(2) and (3) On temporary and reduced salaries provided until April 1, 1963 by Wm. S. Merrill & Co.

(4) and (5) On full time during summer months and part time the remainder of the year.

VII. CURRICULUM VITAE

CARL G. HELLER, M.D., Ph.D.
PRINCIPAL INVESTIGATOR

PRIVACY ACT MATERIAL REMOVED

Degrees: 1936 - Ph.B. - Zoology - University of Wisconsin
1940 - M.D. - Medicine - University of Wisconsin
1940 - Ph.D. - Physiology - University of Wisconsin

Honors: Travel award to Oxford for the International Physiological Congress, 1947
Ciba Award in Endocrinology, 1948
Squibb Award of the Pacific Coast Fertility Society, 1962

Experience: 1940-41 Internship, Wisconsin General Hospital
1941-44 Residency training, City of Detroit Receiving Hospital, Internal Medicine.
1941-42 Fellowship, Wayne State University
1942-43 American College of Physicians Fellowship
1942-44 Instructor, Wayne State University, Internal Med.
1944-45 Assistant Professor, Wayne State University Physiology
1945-48 Associate Professor, University of Oregon Medical School, Physiology and Internal Medicine
1948-57 Head, Division of Endocrinology and Associate Professor of Medicine, University of Oregon Medical School.
1958-on Head, Division of Reproductive Physiology, Pacific Northwest Research Foundation.

Selected

Publications: Heller, C.G., H. Lauson, and E. L. Sevringhaus. The Immature Rat Uterus as an Assay End-Point for Gonadotropic substance. 1938. Am. J. Physiology. 121:364.

Heller, C. G. and E. J. Heller. Gonadotropic Hormone: Urine Assays of Normally Cycling Menopausal, Castrated and Estrin Treated Human Females. 1939. J. Clin. Invest. 18: 171.

Heller, E. J., C. G. Heller, and E. L. Sevringhaus. Gonadotropic Hormone. 1941. Endocrinol. 29:1.

Heller, C. G., C. A. Paulsen, G. E. Mortimore, E. C. Jungck, and W. O. Nelson. Urinary Gonadotropins,

Spermatogenic Activity, and Classification of Testicular Morphology -- Their bearing on the Utilization Hypothesis. 1952. Annals of the New York Academy of Sciences. 55: 685-702.

Heller, C. G., & W. O. Nelson. Hyalinization of the Seminiferous Tubules Associated with Normal or Failing Leydig Cell Function; Discussion of Relationship to Eunuchoidism, Gynecomastia, Elevated Gonadotrophins, Depressed 17-ketosteroids and Estrogens. 1945. J. Clin. Endocrinol. 5:1.

Heller, C. G., D. J. Moore, C. A. Paulsen, W. O. Nelson, & W. M. Laidlaw. Effects of Progesterone and Synthetic Progestins on the Reproductive Physiology of Normal Men. 1959. Federation Proc. 18: 1057-1064.

Rigas, D. A., C. A. Paulsen, and C. G. Heller. Purification of Gonadotrophins Derived from Urine and Pituitary Glands of Human Beings: Observations on Their Electrophoretic Behaviour and Biological Activity. 1958. Endocrinol. 62: 738-748.

Heller, C. G. & W. O. Maddock. The Use of Androgens in Men. 1948. Bul. of the New York Academy of Medicine. 24: 3: 179-194.

Heller, C. G., D. J. Moore, & C. A. Paulsen. Suppression of Spermatogenesis and Chronic Toxicity in Men by a New Series of Bis(dichloroacetyl) Diamines. 1961. Toxicology & Applied Pharmacology, 3: 1: 1-11.

Heller, C. G. & W. O. Nelson. The Testis-Pituitary Relationship in Man. 1948. Recent Progress in Hormone Research. 3: 229.

Heller, C. G., L. J. Matson, D. J. Moore, & Y. Clermont. Rate of Spermatogenesis in Man Determined by Incorporating Tritiated Thymidine Into Testes. 1962. Proceedings of the International Symposium on the Effects of Ionizing Radiation on the Reproductive System. (in press)

Heller, C. G., C. A. Paulsen, & D. J. Moore. Alteration in Spermatogenesis of Normal Men with Synthetic & Natural Progestins. 1960. Proceedings of First International Congress of Endocrinology. Copenhagen Session VIII 1, No. 465.

Heller, C. G. & Y. Clermont. Spermatogenesis in Man:
An Estimate of Its Duration. 1963. Science (submitted
for publication).

Heller, C. G. & D. J. Moore. Dichotomy Between Total
Gonadotrophins (HPG) and ICSH Excretion Produced by
Clomiphene. 1963. Clinical Research 11: 111(abstract)

CURRICULUM VITAE

HARRY JOHN CHRISTOFFERS
SENIOR INVESTIGATOR

PRIVACY ACT MATERIAL REMOVED

Degrees: 1943 Bachelor of Science in Chemistry (cum laude)
University of Washington, Seattle, Washington
1945 Master of Science (chemistry) University of
Washington, Seattle, Washington
1962 Doctor of Philosophy (chemistry), Clark University
Worcester, Massachusetts.

Honors: 1943 Elected to membership in Phi Lambda Upsilon
1943 Elected to membership in Phi Sigma
1943 Elected to associate membership in the Society
of Sigma Xi.
1949 Elected to full membership in the Society of
Sigma Xi.
1950 Awarded an Atomic Energy Commission predoctoral
fellowship.
1955 Awarded a Clark University fellowship (graduate)

Experience: 1944 Assistant in Chemistry, Columbia University
1947 Assistant Instructor in chemistry University of
Kansas
1948 Instructor in chemistry, University of North Dakota
1952 Instructor in chemistry, University of Massachusetts
1957 Assistant Professor of Physical and Inorganic
chemistry, Montana School of Mines
1962 Oceanographic Chemist, University of Washington

Publications: H. J. Christoffers, G. H. Cady, and E. C. Lingafelter,
"The Physical Properties and Crystal Structure of C₆F₁₂"
Journal of the American Chemical Society 69: 2502 (1947).

H. J. Christoffers and G. Kegeles, "The Diffusion Co-
efficients, Viscosities, and Densities of Aqueous Solution
of Acetamide", The Journal of Physical Chemistry (in
press).

CURRICULUM VITAE

JEAN FIELDING
SENIOR INVESTIGATOR

PRIVACY ACT MATERIAL REMOVED

Degree: 1942 Bachelor of Arts in Romance Languages,
University of Illinois

Honors: 1939 Scholarship, University of Chicago
1942 Graduate fellowship, University of Illinois
1960 Graduate fellowship in Genetics, University of
Washington

Experience: 1942 Instructor, University of Illinois
1942 Disbursing Officer, U.S.N.
1946 Instructor, University of Illinois
1957 Engineering aide, Boeing Company

CURRICULUM VITAE

RODERICK T. ROSCOE
RESEARCH ASSISTANT

PRIVACY ACT MATERIAL REMOVED

Degrees: 1954 B.A. - Biology - Whitman College
University of Washington School of Medicine
1954 - 1957

Experience: 1956 Washington State Health Department, Epidemiology.
1958-on Pacific Northwest Research Foundation.

Publications: Paulsen, C. A., Moore, D. J., Roscoe, R. T., and Heller,
C. G.: Failure of Progesterone administration to depress
urinary gonadotrophin excretion in normal menstruating
women. Acta Endocrinologica, 35 (Suppl. 51): 203, 1960.

Moore, D. J., Roscoe, R. T., Heller, C. G. and Paulsen,
C. A.: Failure of Progesterone to depress urinary
gonadotropin excretion in normal menstruating women. in
Human Gonadotropins, ed. by A. Albert, Springfield,
Illinois, Charles C. Thomas, 1961, p. 233-239.

Moore, D. J., Roscoe, R. T., Matson, L. J., and Heller,
C. G.: Increased gonadotrophin excretion induced by
antispermatogenic agents. Clinical Research, 10(1):
88, 1962.

C. G. Heller, R. T. Roscoe, L. J. Matson and D. J. Moore:
Observations on gonadotropin, estrogen, and 17-ketosteroid
excretion, and either spermatogenesis or menstruation
in normal men and women receiving Delalutin. Squibb
Symposium on Delalutin in Advanced Endometrial Cancer
in Women. (in press)

CURRICULUM VITAE

MAVIS J. ROWLEY
RESEARCH ASSISTANT

PRIVACY ACT MATERIAL REMOVED

2

Degrees: 1961 B.S. with major in biology, minor in chemistry
in medical technology from Pacific Lutheran
University.
1962 ASCP registry in histology (Training Swedish
Hospital).

Honors: Undergraduate scholarships - Carl Raymond Gray -
Union Pacific Railroad, Captain Carl Beard Trophy,
Scholarships - National Honor Society Scholarship,
Women of the Moose Science Scholarship.

Experience: 1960-61 Clinical histology - Swedish Hospital
1961-62 Electron microscopy - Swedish Hospital
Histochemistry.
1962-on Pacific Northwest Research Foundation

CURRICULUM VITAE

PRIVACY ACT MATERIAL REMOVED

GEORGE HERBERT NEEDHAM
MICROSCOPIST

- Degrees: 1922 Pharmaceutical chemist
1923 Bachelor of Science in Pharmacy (cum laude)
1924 Master of Science in Pharmacy
Above degrees from the University of Washington, Seattle.
- Honors: 1923-24 Arthur A. Denny Graduate Fellow in Pharmacy
University of Washington.
1925 Elected to full membership in the Society of
Sigma Xi.
1926 Elected a Fellow of the New York Microscopical
Society.
1927 Elected a Fellow of the Royal Microscopical
Society.
1931-32 Served as President of the New York Microscopical
Society.
1946-50 Served as President of the San Francisco
Microscopical Society.
- Experience: 1924-28 Research Chemist and Microscopist, Laboratories
E. R. Squibb & Sons, Brooklyn, New York.
1929-32 Chief Control Chemist, The Hills Brothers
Company, Brooklyn, New York.
1933-35 Own Laboratory of Microscopy and Photomicrography
San Francisco, California.
1936-46 Research Microscopist and chemist, California
Packing Corporation, San Francisco, California.
1947-55 Consultant and Teacher, Microscopy and Photo-
micrography. Two evening courses in these sub-
jects were given at the University of California Medi-
cal School, San Francisco, in 1950 and 1952.
1956-62 Consultant and Teacher, Microscopy and Photo-
micrography, Seattle, Washington.
1956-57 Six months in laboratory of Dr. John L. Bakke,
Veterans Administration Hospital, Seattle, doing
microscopical research on the human thyroid gland.
1958-59 Six months in the laboratory of Dr. Richard
Blandau, Department of Anatomy, University of
Washington Medical School, doing microscopical
research on chick heart muscle.

1960 September to November - Course in Photomicrography
University of Washington, Dental School.

1962-on Research microscopist in laboratory of Dr. Carl
Heller, Pacific Northwest Research Foundation.

Publications: 1958 The Practical Use of the Microscope, Including
Photomicrography, Charles C. Thomas, Publisher.

1962 Hints on 35 mm Colour Photomicrography, especially
with the Leica Camera, Journal Royal Micro-
scopical Society, 80: 235-242.

CURRICULUM VITAE

JOHN L. HOWIESON, M. D.
RADIOLOGIST

Degrees: 1950 Bachelor of Arts (chemistry), University of Kansas
1951 Master of Arts (Anatomy), University of Kansas
1955 Doctor of Medicine, University of Kansas

Experience: 1956 Internship U.S.P.H. Hospital, Brighton, Massachusetts
1957 Armed Forces Special Weapons Project course in
radiation physics, chemistry and biology.
1958 Residency in radiology, University of Oregon.
1962 Diplomat American Board of Radiology.

CURRICULUM VITAE

E. KENNETH VOLLMAR, M.D.
RADIOLOGIST

Experience: Licensed to practice medicine in Oregon.
Chief of Radiology at Salem Memorial Hospital.
Diplomat American Board of Radiology.

CURRICULUM VITAE

YVES CLERMONT, Ph.D.
CYTOLOGIST

Experience: Professor of Anatomy, McGill University, Montreal
P.Q., Canada

CURRICULUM VITAE

STEPHEN C. HELLER
TECHNICIAN

PRIVACY ACT MATERIAL REMOVED

Degree: 1962 Bachelor of Arts (Zoology) University of
Washington

Honors: 1961 Saiyuk Society (Service & Leadership at
University of Washington)

Experience: 1958-on Technician, Pacific Northwest Research
Foundation, Seattle, Washington.

CURRICULUM VITAE

GARY V. HELLER
TECHNICIAN

Education: Junior in biology, Seattle University
Seattle, Washington

Experience: 1959-on Technician, Pacific Northwest Research
Foundation, Seattle, Washington.

VIII. FACILITIES AVAILABLE

A. PACIFIC NORTHWEST RESEARCH FOUNDATION, SEATTLE

The Reproductive Physiology Division has newly constructed laboratories in the former nurses' dormitories comprising a total of 1,760 square feet. These include (1) a completely equipped histology laboratory, (2) a microscopy room, (3) dark-room for radio-autography, (4) small animal room, (5) hydrolysis and extraction room for steroids, (6) steroid analysis laboratory, (7) large general laboratory used for gonadotropin analysis, rat autopsy and general chemical analysis, (8) small office. These are for our exclusive use and do not include joint facilities of the Foundation.

A fully equipped chemical laboratory has been assigned to us in the new wing now under construction and is to be completed in 9 months. The area comprises 760 square feet.

B. OREGON STATE PENITENTIARY, SALEM

At the Oregon State Penitentiary we have available a completely equipped modern operating room, scrub room, sterilizing equipment, and hospital beds. (All biopsy and vasectomy subjects are hospitalized for 24 hours or more.) A complete (inmate) staff of nurses, orderlies, surgical, laboratory and X-ray technicians are at our disposal. A well-equipped routine laboratory is available for performing sperm counts, making sperm smears, conducting urinalyses, and performing hemotological studies. In addition, liver function tests, blood sugar, electrolyte studies, etc. are conducted as needed. We own two

refrigerators used for urine collecting and four specially designed refrigerator boxes for shipping urine between Salem and Seattle. Rooms for physical examination and interviews are available. A pharmacy and X-ray laboratories are also available.

C. THE TUMOR INSTITUTE OF THE SWEDISH HOSPITAL, SEATTLE

Dr. Orliiss Wildermuth, Director and Chairman of the Isotope Committee, is our sponsoring agent for the radioactive thymidine portion of the program. Mr. Peter Wootton, Radiation Protection Officer and Physicist, will make appropriate dilutions of the tritiated thymidine and will make provisions for its storage, calibration and disposal.

D. OFFICE AND SMALL LABORATORY OF DR. CARL HELLER, PORTLAND

The office and small laboratory of Dr. Carl G. Heller in Portland, Oregon, 1920 N.W. Johnson serves for the collation of data. Here charts of inmates are typed and all data assembled, initial preparation of tissues is performed, sperm counts rechecked, manuscripts typed, correspondence conducted, etc.

E. MAJOR PERMANENT EQUIPMENT AVAILABLE, SEATTLE

Major permanent equipment available includes the following: A Zeiss Model WL binocular research microscope with built-in light source and Reichart view screen for group study; a complete micro-technique set-up including an A.O. Model 820 microtome, paraffin imbedding oven, etc.; a Volland analytic balance, triple beam balances, and a Roller-Smith torsion balance for bioassay purposes; Spincro Duostat and Durrum electrophoretic cell; Spincro automatic speed filter, International centrifuge, and Beckman Zeromatic pH meter for gonadotropin

processing; Kahn-type automatic shaker, tube heater, battery charger and Beckman Model B spectrophotometer, water baths, hot plates, drying oven, automatic pipet washer and continuous-flow extraction apparatus for steroid determinations; refrigeration facilities for storing urine and seminal fluid specimens; biopsy packs and an autoclave for their sterilization; a Monroe calculator and electric typewriter.

IX. REFERENCES

1. a. Heller and Moore, Clin. Res., 11, 111 (1963).
b. Heller, Moore, and Paulsen, Toxicology and Applied Pharmacology, 3, 1(1961).
2. Y. Clermont, Am. J. Anat., in press.
3. Heller, Matson, Moore and Clermont, "Rate of Spermatogenesis in Man Determined by Incorporating Tritiated Thymidine into Testes" in "Proceedings of the International Symposium on the Effects of Ionizing Radiation on the Reproductive System", in press.
4. Heller and Clermont, Science submitted for publication 1963.
5. E. F. Oakberg, Radiation Res., 2, 369 (1955).
6. Robinson and Engle, J. Urology, 61, 781 (1949).
7. P. S. Henshaw, J. Natl. Cancer Inst., 4, 485 (1944).
8. E. F. Oakberg, Am. J. Anat., 99, 391 (1956).
9. E. F. Oakberg, J. Morphol., 97, 39 (1955).
10. E. F. Oakberg, Radiation Res., 11, 700 (1959).
11. Albers-Schonberg, Munch. Med. Wochensch, 50, 1859 (1903).
12. V. Monesi, Radiation Res., 17, 809 (1962).
13. E. C. Roosen-Runge, Biol. Rev., 37, 343 (1962).
14. Roosen-Runge and Barlow, Am. J. Anat., 93, 143 (1953).
15. J. A. Pitcock, "Late Testicular Lesions in Irradiated Monkeys" in "Proceedings of the International Symposium on the Effects of Ionizing Radiation on the Reproductive System", in press.
16. Maddock, Epstein and Nelson, Ann. N.Y. Acad. Sci., 55, 657 (1952).
17. A. Albert, Proc. Staff Meet., Mayo Clinic, 30, 552 (1955).

18. Heller, Lauson, Sevringhaus, Am. J. Physiol., 121, 364 (1938).
19. McArthur, Worcester, Ingersoll, J. Clin. Endocrinol., 18, 1186 (1954).
20. Lauson, Heller, Golden and Sevringhaus, Endocrinol. 24, 35 (1939).
21. Dreker, Heisler, Scism, Stern, Pearson, and McGavack, J. Clin. Endocrinol. & Metabolism. 12, 55 (1952).
22. Ichii, Forchielli, Perloff and Dorfman, Anal. Biochem., in press.
23. M. Finkelstein, Acta Endocrinol., 10, 149 (1952).
24. Gurdip, Angers, vande Wiele and Lieberman, J. Clin. Endocrinol. Metabolism, 22, 935 (1962).
25. J. B. Brown, Biochem. J., 60, 185 (1955).
26. Finkelstein, Forchielli, and Dorfman, J. Clin. Endocrinol. Metabolism, 21, 98 (1961).
27. Forchielli, Sorcini, Nightingale, Brust, and Dorfman, Anal. Biochem. Submitted 1962.
28. K. Savard, J. Biol. Chem., 202, 457 (1953).
29. Futterweit, Narcus, McNiven, and Dorfman, Biochem. Biophys. Acta. To be submitted.
30. H. H. Wotiz, "A Short Course in Gas Chromatographic Analysis of Urinary Steroids", Boston University Medical Center 1963.

B. PERMANENT EQUIPMENT REQUIRED

Radiation X-ray therapy unit, 120-140 KVP range	
Dosimeter	
Lead shielding - portable	
Shielding and holding device for testes	\$3,000
Histological Japanese microscope	900
Steroid Studies	
Gas Chromatograph with accessories	12,000
Fluorimeter	1,100
Fraction collector, Gibson type	1,200
Flash evaporator	300
Incubation oven	900
Thin-plate equipment	500
Automatic sample changer for scintillation counting of radioactive materials (Major portion of the counting equipment already owned by Dr. Bakke of Foundation and would be shared by the two groups.)	3,800
	<u>TOTAL</u>

C. SUPPLIES AND SERVICES

Gonadotropin Assays	
Female rats for bioassay	1,000
Hypophysectomized male rats for bioassay	1,500
Chemicals, glassware, animal feed	1,000
Histological - chemicals, stains, slides, slide boxes, etc.	600
Steroid	
Glassware and other general laboratory equipment. Custom made "Preedy" and "Gurpide" chromatographic columns and phase changers; separatory funnels; equipment for acetylation and bromination of steroids before final chromatography; microsyringes, etc.	1,200
Chemicals	
Enzymes for hydrolysis; solvents; purified natural or synthetic steroids; radioactive steroids; dry ice for trapping eluted fractions; brominating and acetylating reagents; etc. (a much greater expenditure is necessary during the establishment of proper procedures than would be required to carry them out later on a routine basis.)	2,500
	<u>TOTAL</u>
	<u>\$7,800</u>

D. TRAVEL

For Dr. Clermont, Montreal-Seattle

For two Seattle Investigators, Seattle-Montreal

For additional trips to Salem

TOTAL

E. COMMUNICATION

Telephone - For Seattle to Portland and Salem calls.
weekly calls to Madison and/or Chicago for
placing assay rat orders. Misc. calls

300

Publications - reprints, photographs and lantern slides

600

Freight charges for iced refrigerator boxes of urine

400

TOTAL

\$1,300

F. PENITENTIARY COSTS

Postage, chemicals and equipment

300

Payment to 40 inmates at \$200 each

8,000

TOTAL

\$8,300

G. INDIRECT COSTS*

(Indirect costs are calculated at 15% of all items
administered by the Foundation, except permanent equipment.
This also excludes \$8,700 administered by the Oregon State
Penitentiary (\$8,300 penitentiary costs plus \$400 to
Dr. Vollmar.)

\$8,743

TOTAL

\$8,743

* It is the policy of the Pacific Northwest Research Foundation
to follow National Institutes of Health rules regarding
indirect costs.

II. SUMMARY

A. SALARIES	
B. PERMANENT EQUIPMENT REQUIRED	23,700
C. SUPPLIES AND SERVICES	7,800
D. TRAVEL	
E. COMMUNICATIONS	1,300
F. PENITENTIARY COSTS	8,300
G. INDIRECT COSTS	<u>8,743</u>

GRAND TOTAL

REPOSITORY: DOE-RICHLAND
COLLECTION: GSS HUMAN TEST SUBJECTS STUDIES
PRISONER STUDY

BOX: 046264

FOLDER: 1780 - HELLER PACIFIC NW RES. FOUNDA.

ASSIGNED NUMBER: RLHTS 94-0049

3002258.A