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BIOCHEMICAL STUDIES RELATING TO THE EFFECTS OF
RADIATION AND METALS

by

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Abstract

Various biochemical effects of radiation have been studied in 10 patients given total body x-ray in total doses of 75 to 310 r (skin), in 5 patients given 20 to 36 mc. P^{32} , and in 5 project personnel accidentally exposed to excessive doses of external radiations. Similar studies have also been made of several hundred Plutonium Project personnel exposed in most instances to relatively small amounts of uranium, lead and other metals, and to chemicals and radioactive sources. These studies included the investigation of liver function, of white blood cell chemistry, and of various urinary constituents such as coproporphyrin, urobilinogen, "corticosteroid-like" substances, catalase, and uranium.

Definitely excessive exposures resulted in various abnormalities in the above tests. With the exception of urinary uranium analysis none of them are specific in indicating overexposure to either radiation, metals, or chemicals. When interpreted in the light of the exposure and medical history, however, it is felt that they may aid in the clinical interpretation of relatively marked overexposure at least. In addition, they may afford further insight into the nature of radiation effects.

The literature on the biochemical effects of radiation is reviewed briefly, and certain suggestions are offered regarding future investigations in this field.

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Biochemical Studies Relating to the Effects of Radiation and Metals

1. Introduction.

The biochemical detection of incipient damage resulting from exposure to radiations or to toxic materials has become increasingly important in recent years. This was especially true on the Manhattan Project because of the well known development of late insidious and "irreversible" changes such as anemia and malignancy in individuals with chronic radiation exposure. Unfortunately, however, the successful solution of this problem has been hampered by our lack of basic information on the early toxic effects of these materials.

The known chemical and biochemical effects of radiation have been reviewed in recent years by a number of investigators⁽¹⁻¹¹⁾. A brief survey of the field along with studies of experimental animals in the Plutonium Project is included elsewhere in these volumes⁽¹²⁾. Also included in these volumes are reviews of the effects of radiations on chemicals⁽¹³⁾, enzyme systems⁽¹⁴⁾, immune reactions⁽¹⁵⁾, clotting mechanisms⁽¹⁶⁾, and hemoglobin metabolism⁽¹⁷⁾. These will therefore not be considered in any detail here.

The studies reported in the literature have indicated that a number of biochemical systems may be affected by therapeutic doses of radiation. These involve the metabolism of carbohydrates⁽¹⁸⁻²²⁾, nucleic acids and purines^(6,23-29), proteins and protein derivatives^(1,30-32), electrolytes⁽³³⁻⁴⁴⁾, cholesterol⁽⁴⁵⁻⁵³⁾, histamine-like substances^(9,54-58), and other compounds. The relation of these effects to acid-base balance⁽⁵⁹⁻⁶⁶⁾, excretion of specific proteinases in urine⁽⁶⁷⁻⁶⁹⁾, and diminished intestinal absorption^(60,71) have also been described.

Though these reported studies have permitted some insight into the nature of radiation effects, very few seemed sufficiently sensitive or

specific to be useful in detecting early damage to project personnel. A series of excursions into various fields of metabolism were therefore undertaken in the hope that one at least would yield clues for further and more comprehensive investigation.

Essentially, two problems were considered within the scope of our studies. The first involved an attempt to determine the earliest and most sensitive changes possible in experimental animals and human subjects administered known amounts of radiation and metals. The second involved an attempt to evaluate the significance of the changes found both in experimental subjects and in project personnel.

Our studies on effects of radiation^(17,72-77) and metals⁽⁷⁷⁻⁸⁰⁾ in experimental animals are reported elsewhere in these volumes. The present report is concerned only with investigations of project personnel and of hospital patients treated with total body x-irradiation or with P³². The clinical and exposure histories of the various groups studied are considered in the experimental section.

It should be pointed out that the interpretations of the studies in the few patients available to us were generally complicated by previous clinical abnormalities. In addition, conditions were such that we had little choice as to the type of patient studied or to the time of study. The extent of some of the investigations is therefore limited.

The complicating factors in personnel were even more serious: exposures were usually multiple (i.e. to chemicals, metals, and radiation), and minimal in amount. In addition serious consideration had to be given the previous exposure record, the past and present clinical histories and personal habits such as hours of work (some personnel would often go several days with only a few hours sleep), and in a few instances, alcohol consumption.

Despite these limitations, it is felt that a number of significant observations have been made which have not been previously reported. In many instances, as noted in the text, these observations have been confirmed in the better controlled animal experiments.

2. Materials and Methods.

2.1 Tests Performed.

The tests chosen were intended to cover as wide a range of functions as practicable. At the same time it was hoped to keep them sufficiently simple to permit their application on a fairly large scale with the least possible inconvenience to personnel. Thus, for example, 15 ml whole blood and a single sample of urine usually sufficed for all the studies to be described. As noted in the experimental section, however, not all tests were done on each of the subjects.

a. Porphyrin Metabolism.

(1) Urinary Excretion of Coproporphyrin.

The analytical procedure used, as well as studies of the relation of erythropoiesis and of experimental metal and radiation exposure to the excretion of urinary coproporphyrin are considered elsewhere in these volumes^(72,78,81,82). Lead exposure and various types of liver dysfunction especially are known to be associated with elevated values. Large doses of x-irradiation, on the other hand, resulted uniformly in diminished porphyrin excretion in treated dogs. Where lethal doses were given, values rose appreciably during the last few days of life.

(2) Erythrocyte Protoporphyrin.

The method of Grinstein and Watson⁽⁸³⁾ was used for erythrocyte protoporphyrin determination. One slight modification was used: the blood and acetic: ethyl acetate solution was ground in and filtered directly through a sintered glass funnel instead of being ground in a mortar

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and filtered through filter paper.

Lead poisoning and iron deficiency anemia especially are known to be associated with elevated values^(84,85).

b. Liver Function.

The serum cephalin cholesterol flocculation test of Hanger⁽⁸⁶⁾ and the serum colloidal gold test of Gray⁽⁸⁷⁾* were employed along with the urinary urobilinogen test of Watson⁽⁸⁸⁾. More recently, the thymol turbidity test of MacLagen⁽⁸⁹⁾ has also been used.

These studies were emphasized despite their lack of etiological specificity because we felt they offered good screening reactions which might pick up evidence of overexposure to a large variety of materials.

The cephalin cholesterol test was modified by the use of serial dilutions of serum before addition of the antigen, as described elsewhere⁽⁷⁷⁾. Results reported here, however, are all based on the first tube which is identical to that described by Hanger.

c. Excretion of "Corticosteroid-like" Substances.

These studies were stimulated by a recent report by Talbot et al⁽⁹⁰⁾ on the increased excretion of certain "corticosteroid-like" compounds in patients with hyperfunction of the adrenal cortex. Especially high values were found in patients with severe thermal burns.

Our studies of these compounds were limited almost entirely to the Los Alamos group. Because all but 1 of the urine samples became available just as our group was disbanding in the spring of 1946, exhaustive studies were impossible. Instead, the urine was partially fractionated as described by Talbot et al and studied spectrophotometrically, using a Beckman spectrophotometer. Special emphasis was given the spectrum from 220 to 350 mμ.

* We are indebted to Doctor Gray for assistance in preparing the colloidal gold.

The procedure used involved extraction of a 50 to 100 ml. portion of urine with chloroform 3 times. The chloroform was washed 3 times each with water and 0.1 normal NaOH. These washes were discarded. The CHCl_3 was next evaporated to dryness under a partial vacuum, and the residue was dissolved in benzene. The benzene was extracted 3 times with water and the combined water was extracted 3 times with chloroform. The latter was evaporated to dryness under partial vacuum. The residue was dissolved in methyl alcohol for spectrophotometric analysis.

All reagents were freshly distilled before use.

d. Excretion of Urorosein and other Pigments or Chromogens.

Urorosein is a red compound formed in strong acid solution by the oxidation of indolacetic acid. Its exact clinical significance is unknown, but its excretion appears to be most commonly associated with dietary deficiency⁽³¹⁾.

The following scheme was used to extract these compounds prior to analysis.

Fraction (A) is the so-called "unoxidized" fraction. Fraction (B) is the so-called "oxidized" fraction.

The absorption of each fraction (A and B) was quantitated on the Evelyn photoelectric colorimeter using first a 400 mμ and then a 520 mμ filter*. Each solution was next examined spectroscopically with a Zeiss spectroscope and the presence of absorption bands noted. In isolated cases the Beckmann spectrophotometer was used to prepare absorption spectra from about 400 mμ to about 600 mμ.

It should be emphasized that urobilin and numerous other pigments, mostly unidentified, are also included in this fraction. The quantitative procedure is therefore not at all specific for urochrome, although the absorption band at 543 mμ appears to be fairly specific.

e. Urinary Excretion of Catalase.

Catalase was determined by an iodometric titration procedure, as described elsewhere⁽⁸⁰⁾.

f. Urinary Excretion of Uranium.

The microfluorometric method devised for the determination of uranium in 0.1 to 0.05 ml of urine is described elsewhere in these volumes (92,93).

g. White Blood Cell Chemistry.

These studies were stimulated largely by the report by Abels et al⁽⁴¹⁾ of an increase in acid-soluble phosphorus in white blood cells of leukemia patients given subtherapeutic doses of x-ray.

Two methods devised for the quantitative isolation of morphologically intact white cells from venous blood free of other cellular elements

* Though urochrome has an absorption band maximum at about 543 mμ as viewed spectroscopically, its absorption on the Evelyn colorimeter, was found to be greater with a 520 mμ filter than with a 540 mμ filter. This is due to the relatively greater total absorption at the shorter wavelengths as compared to those above 543 mμ.

have been described elsewhere in these volumes^(94,95). Since these chemical studies were done before development of the gramicidin- lysolecithin method, only saponin treated cells were used in these studies.

Details of the analytical procedures used and experimental results obtained are described elsewhere⁽⁷⁶⁾. Chemical analyses included the following:

1. Phosphorus determinations were done on all fractions using the method of Gomori⁽⁹⁶⁾. The more sensitive method of Berenblum and Chain⁽⁹⁷⁾ was used in our initial studies, but was discarded because of its greater complexity.

2. Pentose determinations were done in most of the studies. Ribose was estimated by the method of Mejboune⁽⁹⁸⁾, while the method described by Dische⁽⁹⁹⁾ was used for determination of desoxyribose.

Ultra violet absorption spectra were prepared using a Beckman spectrophotometer.

2.2 Scoring System and Group Classification.

To aid in interpretation of some of the data, a system of scoring was used in which results of several of the tests were considered in terms of 0 to 4 plus scores. A score of 0 indicated a result which appeared to be definitely within the normal range. A score of 1 plus was questionably elevated, and scores of 2 to 4 plus were increasingly elevated above the usual control values. It should be emphasized, however, that scores of 3 or 4 plus in project personnel were rarely associated with clinical complaints.

The range of values corresponding to each of the scores used are set forth in Table 1.

Control subjects were obtained chiefly from the following 3 groups: (1) Medical students at the University of Chicago; (2) Veterinary students at Iowa State College, Ames, Iowa; (3) Project personnel believed to have had negligible exposure to metals, chemicals, or to radiation, and found to be in good physical conditions.

Project personnel were classified according to their major exposure. This was done following personal interview to obtain as complete as possible previous clinical and exposure histories. Where indicated, this was supplemented by further discussion with the proper supervisors.

3. Experimental

3.1 Studies of Hospitalized Patients Given Total Body X-ray.

Six hospitalized patients were given a total effective dose of 75 to 310 r (skin) of 200 KV x-rays to total body in divided doses. The therapy was given to 3 patients for neoplastic disease, to 2 patients for partially controlled polycythemia with elevated leucocyte counts and to 1 patient for chronic arthritis.

The details of the treatment are described elsewhere⁽¹⁰⁰⁾, but briefly stated they are 200 KV with a half value layer of 1.5 mm Cu, (usually) 3.55 r/minute measured on the skin. The anterior surface of the body was covered by 2 fields and the posterior by 2 fields. Each surface was assumed to receive what each field received, but the "total body dose" was a sum of that received by the anterior and posterior surfaces.

Case I. Most complete studies were made of a 58 year old female (M. G.) who received a total dose of 310 r x-ray to the surface of the body over a period of 18 days. The parotid tumor for which she was treated was first noted in 1929. It was excised in 1934, and again in 1939, 1941 and 1944. Radiation therapy (dose unknown) was given following the first excision. At the time of surgery in April, 1944, the tumor had reached "volley"

ball" size. Pulmonary metastases were believed to be present. The patient had suffered a 40 pound weight loss during the preceding 18 months.

Treatment was divided into a series of almost daily doses of 20 to 25 r total body x-ray. This was discontinued on the nineteenth day. Supportive therapy included vitamins B and C, feosol, barbiturates, aspirin, and morphine. The latter were given before, during, and after the x-ray therapy.

a. Hematology.

The most striking hematological effect of the treatment was a pronounced shift to the left in the Arneht count, with an increase on about the fifth day in the ratio of 1 and 2 lobed polymorphonuclear cells as compared to 4 and 5 lobed cells. The lymphocyte count fell gradually, while the polymorphonuclear cells decreased abruptly on about the twenty-fifth day. The platelet count fell temporarily at the end of the first week, rose, and then fell again 2 weeks later. These data are summarized in Figures 1 and 2.

Erythrocyte sedimentation was already increased before therapy was begun. Eight control determination ranged from 40 to 50 mm in 1 hour. After treatment most values ranged from 50 to 57 mm in 1 hour.

b. Urine Chemistry.

Marked increases were found during the last week or two in excretion of urinary coproporphyrin, urobilinogen, and uroporphyrin-like pigments. The latter substances were not determined during the early radiation period. The data are summarized in Figure 3.

c. Blood Chemistry.

1. White blood cell chemistry.

A prolonged increase of several hundred per cent was found in the concentration of phosphorus in the "adenosine triphosphate" fraction of the white blood cells. This increase appeared before any hematological changes were apparent. The data, summarized in Figure 4, are given in terms

Figure 1. Hematology studies in a patient administered a total (skin) dose of 310 r total body X ray over a period of 18 days (Case I).

Figure 2. The effect of total body X irradiation on polymorphonuclear cell maturity (Case I).

o-----o = 1 and 2 lobe cells
O-----O = 4 and 5 lobe cells

Figure 3. The urinary excretion of urobilinogen, "uroscopin", and coproporphyrin in a patient treated with total body X irradiation (Case I).

----- %/day: ----- 1%

Figure 4. The accumulation of so-called "adenosine triphosphate" phosphorus in the white blood cells of a patient given total body X irradiation (Case I).

of both total phosphorus per million white blood cells and the ratio of this fraction to total acid soluble phosphorus. The latter is included to indicate the relatively specific nature of the increase.

Results of analyses of the various white blood cell fractions are summarized in Table 1. No attempt was made in this study to further fractionate the nucleoprotein and lipoid soluble compounds. Neither were pentose or ultraviolet absorption studies made.

Case II. A 41 year old male with a metastasizing gastric carcinoma was given a total body dose of 105 r (skin) in 15 doses of 7 r each over a 20 day period. He died at the end of this period.

The most striking biochemical change found was an increase of several hundred per cent in the urinary excretion of uropigments. This was most marked in the oxidized fraction. Unlike Case I, the excretion of these compounds returned to the normal range before death. The data are summarized in Figure 5.

The serum cephalin cholesterol reaction was 1-3 plus even before the start of x-ray therapy. On the other hand, 7 determinations of serum colloidal gold both before and after therapy were normal. The excretion of urinary urobilinogen, which was normal before therapy, reached a peak value of 12 mg. per day 4-5 days after treatment was begun.

Two determinations of erythrocyte sedimentation rate before treatment showed values of 21-41, and 21-34 mm. at 30 and 60 minutes respectively. Six and 9 days after beginning treatment, values of 38-49, and 41-47 were obtained.

The excretion of total urinary coproporphyrin fluctuated considerably before therapy, with values as high as 485 micrograms per day. No significant change was apparent after therapy.

The WBC fluctuated from 5600 to 10,200/cmm until 2 days before death when it fell to 2,250.

Figure 5. The effect of total body X ray on the urinary excretion of uroscsein and other urinary pigments in a patient with a metastasizing gastric carcinoma (Case II).
o-----o 520 mμ; e-----e 400 mμ

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Case III. This 73 year old male was given a total (skin) dose of 226 r total body x-ray in 2 doses over a period of 2 days (11-26 and 27, 1943), for treatment of a squamous cell carcinoma of the hand. He was discharged after slightly more than 3 months hospitalization.

Like the previous 2 cases he showed a striking increase in the excretion of uroporphyrin-like pigments.

During the control period, repeated determinations of serum cephalin cholesterol showed elevated values of 3 and 4 plus. After treatment, oddly, values returned to normal; 7 of 8 determinations from 12/9 to 12/22 were 0 or 1 plus and one value was 2 plus. From 1/1 to 3/3 when the patient was discharged values became abnormal again; all 6 determinations during this period were 2 or 3 plus. During this latter period 5 determinations of serum colloidal gold were all 3 or 4 plus.

The concentration of erythrocyte protoporphyrin was determined at 1 to 3 day intervals for about 1 month after administration of x-rays, and at longer intervals thereafter. No significant change was found except during the week before discharge when values of 46 and 62 micrograms per cent were found.

The hematology data on this patient are presented elsewhere⁽¹⁰⁰⁾.

Case IV. A. year old was male with polycythemia vera was given a total (skin) dose of 150 r total body x-ray over a 1 week period. His hematocrit at the time treatment began was only about 40 per cent, but his white blood count was 95,000.

The most striking change observed was an increase in the erythrocyte sedimentation rate. This was especially interesting because the hematocrit fell less than 5 per cent during the period of study. The data are shown in Figure 6.

No significant changes were observed in liver function or in porphyrin metabolism.

Figure 6. The elevation of erythrocyte sedimentation rate in a patient with polycythemia vera treated with total body X ray (Case IV) (Hematocrit fell less than 5% during period of study).

o-----o = 60 minute reading
o-----o = 30 minute reading

The hematology data are described elsewhere⁽¹⁰⁰⁾.

Studies of patients 5 and 6 are discussed elsewhere⁽⁷⁶⁾.

Cases VII-IX. A (skin) dose of 30 r total body x-ray was given to 4 volunteer members of our group. One received daily doses of 2 r, while the others received 3 doses of 10 r each. Chief emphasis was given the study of white blood cell phosphorus in these cases. Such changes as were found in some individuals were not consistently present in all four.

3.2 Studies of Patients with Polycythemia Vera Given P^{32} .

Five patients with polycythemia vera were each given a total dose of 20 to 36 mc P^{32} orally over a period of 1 to 5 weeks. All were ambulatory.

In 2 patients, the sedimentation rate reached peak values of 23 and 40 mm in 1 hour respectively. Twelve control values in these 2 patients were all under 2 mm in 1 hour. In both cases however, the hematocrit fell from control levels of 55-64 per cent to 37 and 40 per cent respectively. No change in either hematocrit or sedimentation rate was found in one patient, while in another the hematocrit fell from 75 to 57 per cent with no change in sedimentation rate.

No significant changes were found in liver function as determined by either serum cephalin cholesterol and colloidal gold tests, or by urinary urobilinogen determinations.

As pointed out elsewhere⁽¹⁷⁾, erythrocyte protoporphyrin concentration was not significantly affected by the treatment. Some correlation was found between the protoporphyrin levels and the color index, as might be expected from other reports⁽⁸⁴⁾; highest values were found associated with low color indices.

The urinary coproporphyrin concentrations were unusually low both before and after treatment. Since only "spot" samples of urine were

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available for analysis, no per diem values could be obtained. Their data are described in section 3.4 -c.

In most instances analyses were made of only total phosphorus and acid-soluble phosphorus in the white blood cells. In all, the average value of the acid-soluble P fell after treatment. The over-all average of 28 control determinations was 0.80 micrograms phosphorus per million cells, while the average of 89 analyses after treatment started was 0.66 micrograms per million cells. Possibly significant increases in the adenosine triphosphate fraction were seen in a few instances. These data are discussed in greater detail elsewhere⁽⁷⁶⁾. Hematology studies are reported elsewhere⁽¹⁰⁰⁾.

3.5 Studies of Los Alamos Personnel with Accidental Radiation Exposure.

In September, 1945, and again on May 21, 1946, accidents in the Los Alamos Laboratory resulted in severe overexposure of personnel to penetrating radiations. One death resulted in each instance. In the latter case clinical changes also occurred in 3 of the survivors. The clinical courses of these patients are described elsewhere⁽¹⁰¹⁾.

Case I. Only a single urine sample, passed the day before death, was available from the first patient. It showed the following abnormalities.

a. Urinary "Steroid" Fraction. The most interesting abnormality was in the ultraviolet absorption spectrum of the so-called "cortico-steroid" fraction. As shown in Figure 7, there was a marked increase in specific absorption at about 230 mμ. In addition, a bifurcated peak was present at about 320 and 330 mμ. Shown in the same figure, for comparison purposes, are spectra from similarly prepared extracts of urine from Case II of the Los Alamos series, from a patient with severe thermal burns

Figure 7. Ultraviolet absorption spectra of extracts of "corticosteroid-like" substances in the urine of two subjects with accidental lethal exposures to penetrating radiations, (1 and 2), of one patient with a lethal thermal burn (13), and of control subjects (6 and 12).

EXTINCTION PER CC OF URINE

which proved lethal 3 days after the first urine sample was obtained (Case 13), and from 2 supposedly normal individuals (Cases 6 and 12). These data are described in more detail below.

b. Urinary Coproporphyrin. A value of 21 micrograms per cent coproporphyrin (252 micrograms per day) was found in this sample. This is at least twice as high as the accepted upper limit of normal.

c. Urinary Urorosein-like Pigments. Beckman analysis revealed increased relative absorption at about 540 mu. The total absorption, as determined with an Evelyn Colorimeter using the 520 mu filter was not markedly increased, amounting to only a 3 plus score. The absorption spectrum for wavelengths of 370 to 580 mu (unoxidized fraction) is shown in Figure 8. The combined medical student urine represents the usual type of spectrum obtained in control individuals, while spectra similar to that of S.B. who was exposed to uranium oxyfluoride were found occasionally in project personnel.

d. Liver Function. The serum cephalin cholesterol reaction before death was 3-4 plus. The thymol turbidity was normal. The urinary urobilinogen was not determined. An intense band at 495, however, was seen in the oxidized urorosein fraction. This absorption was most likely due to urobilin.

Cases 2-9. Death occurred on the 9th day in Case 2. It is estimated that he received a dose of about 900 rep. Cases 3, 4, and 5 received doses of about 100 to 200 rep. Cases 6, 7, 8, and 9 serve essentially as controls, since their exposures were probably all less than 20 rep.

a. Urinary "Cortico-steroid-like" Substances. Chief emphasis was given the study of the so-called "cortico-steroid-like" substances in

Figure 8. Absorption spectra of urinary "urorosein" fraction (un-oxidized)

- (A) = Case I, Los Alamos .
- (B) = Met. Lab. employee with UO_2F_2 exposure (Chicago)
- (C) = Combined urine from (control) medical students

these individuals. The spectra from Cases 2, 3, 4, and 5 all showed plateaus or actual peaks at about 230 m μ similar to, though not as marked as that found in Case 1. In all instances but one this was greatest 5 to 7 days after the exposure. The relative increase in absorption at 230 m μ persisted 1 to 2 weeks in Case 3. In Cases 4 and 5 the values were still abnormal when the study was discontinued 5 and 3 weeks respectively after the exposure. None of the subjects with minimal exposure (Cases 6-9) showed this phenomenon.

Spectra obtained from Cases 2 and 6, as noted previously, are shown in Figure 7.

To illustrate the data more completely, the ratios of total absorption at certain significant wavelengths have been calculated. These ratios, as listed in Table 3, show a clear cut difference between the urine extracts from the individuals with significant radiation exposure and the control subjects. The ratios of the patient with lethal thermal burns are also seen to be similar to those of the subjects with severe radiation exposure.

b. Urinary Coproporphyrin. Case 2 showed the usual terminal increase in total urinary coproporphyrin excretion. (To 451 micrograms per day). The data are summarized in Table 4.

Increased coproporphyrin excretion was found in Case 3 on the third day. A total of 19.8 micrograms per cent, or 362 micrograms per day was excreted on this day. Thirteen other analyses over a period of a month were normal or somewhat elevated (3 values above 150 micrograms per day).

In other cases the per diem excretion of coproporphyrin was normal or depressed. Case 4, for example, had 7 consecutive samples from May 26 to June 4 with concentrations of less than 3 micrograms per

cent. Per diem values during this period averaged 5% micrograms per day.

Only 2 determinations of the ratio of the 2 coproporphyrin isomers were done. Seventy-seven per cent of the total porphyrin in Case II from May 26 to May 28 was the type III isomer. In Case III the combined sample from May 21 to June 3 contained about 95 per cent type III coproporphyrin.

Interpretation of the data in all instances is made difficult by the fact that no control analyses had been made on any of the individuals.

c. Urinary Urobilinogen. Abnormal values were found in only cases II and III. In the former case values rose steadily and reached 6.2 mg per day on May 28-29, the last sample sent us. 6.7 mg urobilinogen were excreted on May 27-28 and 2.6 mg on June 2-3, by Case III.

The other 45 samples analyzed from Cases III-IX all contained 1 mg per day or less.

d. "Urorosein" Fraction. Repeated spectra were prepared from the urine of Case II. These showed an increase in absorption at about 490-495 mμ which was apparently due chiefly to urobilin. The spectra of the oxidized fraction are shown in Figure 9.

e. Fecal Porphyrins. No definitely significant trends were observed. Coproporphyrin concentration ranged in most cases from 100 to 300 micrograms per cent.

3.4 Studies of Plutonium Project Personnel.

a. Subjects. Biochemical studies have been made of personnel from the following sites:

1. Chicago Metallurgical Laboratory and Argonne Laboratory.

The large majority of our studies were made of this group. Exposures were very heterogeneous and, generally, minimal in amount. The relatively light radiation exposure consisted essentially

Figure 9. Absorption spectra of urinary "urorosein" fraction (oxidized) in Case 2 (L. A.).

of radioactive materials and cyclotron and pile sources. Machine shop workers were exposed to uranium, lead, and other metals. Exposure to uranium oxide was relatively heavy in the early days of the project, but had diminished markedly by the time our studies were begun in the autumn of 1943.

Excessive exposure to chemicals such as carbon tetrachloride and trichlorethylene was sporadic. Several employees, however, had had fairly heavy chemical exposure in munitions plants before joining the Metallurgical Laboratory.

2. Iowa State University, Ames, Iowa

Considerable emphasis was given the study of several dozen individuals exposed to UF_6 and to other salts of uranium and other metals at the uranium production plant. Studies were also made of the group working on the chemistry of radioactive materials.

3. Oak Ridge

These studies were limited to 10 individuals believed to have had relatively heavy exposure to uranium. Samples from these subjects were studied through the courtesy of the Manhattan District Medical Office at Oak Ridge.

4. St. Louis Cyclotron

Occasional studies were made of individuals working with the cyclotron at Washington University, St. Louis, Mo.

b. Tests.

1. Urinary Excretion of Uranium.

Detailed studies on the excretion of uranium in urine of project personnel have been reported elsewhere⁽⁹³⁾. These analyses proved especially valuable at the Iowa State College, Ames, Iowa Project where exposure to various uranium salts was among the heaviest in this

country. As shown in Table 5, results correlated well with the known histories of exposure to uranium. Values of over 50 micrograms uranium per liter of urine were found only in individuals with "moderate" or "heavy" exposure; less than 10 micrograms per liter was found in all cases where exposure was only incidental.

Thirty-eight analyses of urine from 25 individuals in the laboratory at the University of Chicago showed no value above 18 micrograms uranium per liter. All but 2 of the samples contained less than 10 micrograms per liter.

Five urine samples obtained from students at the University of Chicago with no known exposure to uranium contained less than 1 microgram uranium per liter, but probably more than 0.1 microgram per liter.

2. Urinary Excretion of Catalase

These studies have been described in detail elsewhere⁽⁸⁰⁾. They were undertaken because of previous reports from the laboratory at Rochester University, New York, that catalase concentration is increased in the urine of rabbits injected with uranium⁽¹⁰²⁾.

Our studies of rabbits injected with uranium confirmed the results of the Rochester group. No significant difference, however, was found in urinary catalase activity of control and exposed project personnel. We found, too, that urine from even control rabbits contains much more catalase activity than does urine from any humans studied, except for those with known kidney disease.

Studies of project personnel are summarized in Table 6 in terms of the difference in volume of the thiosulfate used in the blank and in the incubated urine samples (see reference 80 for details of method).

3. Urinary Excretion of Coproporphyrin.

Control subjects generally excrete less than 10 micrograms coproporphyrin per hundred ml. of urine. Definite increases were found most consistently in two exposure groups, namely those exposed to lead and to uranium oxyfluoride. Results in the group exposed chiefly to uranium oxide and fluoride were variable. This is probably explained in part at least by animal experiments in which it has been shown that injection of a large dose of uranium in rabbits and dogs depresses coproporphyrin excretion, whereas injection of small amounts results in a slow moderate increase in urinary porphyrin excretion⁽⁷⁸⁾. Thus, for example, the individual believed to have had the heaviest exposure in the Iowa State College Project consistently excreted less than 7.5 micrograms coproporphyrin per 100 cc urine. Seven analyses from November, 1943 to July, 1945 averaged 5 micrograms per cent. Nine and ten months after his leaving the work 2 samples showed increased concentration of 10.5 and 11.4 micrograms per cent respectively.

Personal illness proved another source of elevated values. Thus, for example, a urine specimen received from a control subject in the Ames Laboratory was found to contain 47 micrograms per cent coproporphyrin, one of the highest values we have observed in project personnel. Investigation revealed that a few hours after passing this specimen the subject went home complaining of fever (Temperature about 103°F.) and generalized muscle aches. Several weeks later, when this individual was again apparently normal, a normal coproporphyrin value of 5 micrograms per cent was obtained.

Studies of project personnel and other subjects were summarized in the autumn of 1944. The data are given in Table 7.

Attention has already been called to the elevated values found terminally in the 2 subjects with lethal radiation exposure and in the subject with carcinomatosis who died several weeks after receiving radiation therapy. In all three, the elevated coproporphyrin excretion proved a bad prognostic sign. These findings agree with those in dogs given lethal exposure to radiation⁽⁷²⁾.

Values of over 20 micrograms per cent were commonly found in hospital patients suffering from various types of hepatitis.

A variety of studies were undertaken to aid in the interpretation of the coproporphyrin data in project personnel. These included studies of the diurnal variation in porphyrin (and "urorosein") excretion, and of the consistency in porphyrin excretion by the same individual over a period of weeks or months. The data, as summarized in Tables 8 and 9, indicate that concentrations are generally higher in samples passed in the afternoon than in those passed before starting work in the morning. (A similar finding has been reported for urobilinogen excretion⁽¹⁰⁶⁾). They also indicate that considerable variation may occur in successive samples from the same individual. "Control" subjects, however, rarely show the marked increases found in significantly exposed personnel or in "abnormal" subjects.

An Unusual Case of Idiopathic Porphyrinuria: During the course of routine urinary porphyrin studies of personnel at the University of Chicago Laboratory, an unusual case of idiopathic porphyrinuria was discovered. A male chemist, age 20, was found to excrete up to 449 micrograms coproporphyrin per 100 ml. urine, and as high as 65,000 micrograms coproporphyrin per hundred grams of feces. (Upper limit of normal for fecal coproporphyrin is probably about 400 micrograms per cent.) The

type III isomer constituted the large majority of the porphyrin in both urine and feces. This abnormality in porphyrin metabolism could not be attributed to any known previous exposure. A more complete report of this case is given elsewhere⁽¹⁰⁴⁾.

4. Red Cell Protoporphyrin. This data was reviewed in October, 1944. At that time only 20 of 381 individuals studied had an erythrocyte protoporphyrin level above 60 micrograms per cent. Fourteen of these had moderate to relatively "heavy" exposure to metals. Two others had an iron deficiency anemia. One had a "leukemoid" blood picture.

The highest values were found in individuals with histories of exposure to lead. One such individual had been exposed 2 to 5 months previously, before joining the Metallurgical Laboratory, to lead enamel and lead fumes. Increased basophilic stippling in his red cells had been noted at that time. Three determinations of protoporphyrin in his red cells over a 3 week period gave values which ranged from 545 to 748 micrograms per 100 cc, as compared to an upper normal limit of about 40 micrograms per cent. His urinary coproporphyrin concentration was also elevated; values ranged from 14 to 27 micrograms per 100 mls. urine.

Repeated porphyrin studies were made of several individuals with variable exposure to lead at the Argonne Laboratory. Case 1, who was most heavily exposed, also had the greatest increases in erythrocyte protoporphyrin and in urinary coproporphyrin excretion.

The data are summarized in Table 10.

5. Urinary Excretion of Urobilin and other Pigments.

Exposed project personnel tended to excrete a higher concentration of urinary pigments than did our control subjects. No specific etiological factors were noted, however. Absorption bands at 543 mμ were most com-

mon among the chemists. Though most of them worked with radioactive materials, the fact that those with the highest concentrations had the greatest chemical exposures makes it seem likely that the chemical exposures were far more significant than the radiation exposures.

Absorption bands were sometimes seen at 510 mμ, especially in the Chicago group exposed to uranium. It, like the 543 band was most common in the "oxidized" samples. The significance of this band is not known. Watson and Layne⁽⁹¹⁾ described a weak absorption band at 511 mμ in association with the 543 band of uroporphyrin. Because its occurrence has been reported in pigs on a pyridoxine deficient diet⁽¹⁰⁵⁾, nine individuals with positive 510 bands were given 10 mg of pyridoxine hydrochloride daily for 10 days. No significant change occurred in the appearance of this band. Neither was urinary coproporphyrin excretion affected significantly by this treatment.

On standing over night at room temperature, roughly 1/3 of the unoxidized samples in each group and half or more the oxidized samples develop absorption bands at about 495 mμ. The band at 510 disappeared in several oxidized samples after 24 hours, while in the unoxidized samples it became more intense, or became apparent for the first time. The 543 band tended to behave in the same fashion.

These pigment studies are summarized in Tables 11-13.

6. Studies of Liver Function. In only a few instances was it considered possible that abnormal liver function scores might be due to laboratory exposure. Thus, for example, four women exposed to carbon tetrachloride, each had a 2+ cephalin chloesterol reaction, and 3 of the

4 had a 4 or 5+ reaction in the first colloidal gold tube. Since no control study was made of these individuals before exposure was begun, it is not certain that the abnormal results were due to the CCl_4 . Similarly, two individuals who had been exposed to large amounts of uranium oxide had 4+ cephalin cholesterol reactions in the fall of 1943. By this time exposure to uranium was negligible. During the course of the next several months, reactions returned nearly to normal in both instances. Again, we have no data on their liver function prior to exposure to the uranium.

The data up to the summer of 1944 are summarized in Tables 14 and 15. Table 14 includes only those cases where the predominant exposure is relatively clear cut. This selection brings in those individuals who showed roughly half the abnormalities found. Watson's modified semiquantitative procedure⁽¹⁰⁶⁾ was used for the determination of urobilinogen in these studies.

It should be pointed out that the average age of personnel exposed to non-uranium metals was higher than that of the other groups and may possibly account, in part, for the increased incidence of abnormal tests found in this group.

In correlating results of different tests we find that no two give consistently similar results. The extent of correlation is indicated in Table 15. All 3-4+ results are included here, regardless of exposure history. Also included for comparison purposes are the results of porphyrin studies in the same individuals.

Time has not permitted complete clinical evaluation of the serial dilution modification of the Hanger cephalin cholesterol flocculation test. There is no doubt, however, that sera which give the same result with the usual 1 tube test may give entirely different results with the diluted samples. The significance of this is not clear. Several different types of reaction are illustrated in Figure 10.

Figure 10. Reaction patterns obtained using the serial dilution modification of the hanger cephalin cholesterol flocculation test.

c.g. = colloidal gold test

Flocculation

Serial Dilution Tube Number

7. Evaluation of Total Scores. In most instances the difference between exposed project personnel and control subjects was more apparent in the total score than in any single abnormality. It thus seemed that an accumulation of 1 and 2 plus scores might be significant even where none of these scores by themselves were significant. This is illustrated in Tables 16 and 17, modified slightly from one of our early reports in 1944.

4. Discussion.

4.1 General Considerations.

In reviewing the literature on the biochemical effects of radiation, one is struck by the unusual amount of conflicting data presented; almost every blood and urinary constituent studied has been reported variously as elevated, depressed, or unchanged. The reason for this variance is to be found largely in the wide range of experimental conditions, involving differences in type of radiation, total dose, dose rate, amount of body exposed, clinical status of subject at time of radiation, and time of analysis after radiation. Thus, for example, Jacobs and Motojima⁽⁴⁵⁾ report blood cholesterol concentration to be elevated in irradiated patients with carcinoma who are in relatively good clinical condition, whereas it is depressed in cachectic patients. Many reactions are reported to vary as a function of time after radiation. This is illustrated, for example, by studies of alkali reserve which indicate that a transitory acidosis may occur after radiation followed by alkalosis for several days, and then a return of acidosis in the terminal period.

In general the limiting factor in these studies appears to be the fact that the reactions studied are those which appear as a result

of a long chain of interrelated responses; attempts to study more fundamental reactions are relatively few. The studies of Fricke⁽⁴⁾ and others⁽²⁾ on the chemical effects of radiation, of Mitchell^(6;23-25) on nucleic acid metabolism, and of Dale⁽¹⁰⁷⁾ and Barron and others⁽¹⁴⁾ on enzyme metabolism are among the exceptions to this statement.*

While the most immediate concern of our studies was the development of tests which might prove of diagnostic and prognostic value in over-exposed human subjects, the ultimate value of such studies must lie chiefly in the better understanding they might afford of the nature of radiation effect. Such understanding might then lead to more rational therapeutic measures for over-exposure, and possibly to a better understanding of such allied fields as cancer and its treatment.

4.2 Possible Interpretations of Presented Data.

At the outset it should be emphasized that considerable more study is necessary for the proper interpretation of the experimental data presented in the previous section. Certain possible correlations, however, are suggested by the literature.

3. "Corticosteroid-like" substances.

Plasencia⁽⁷⁾ has emphasized certain similarities between radiation toxicity and hypofunction of the adrenals as seen in Addison's disease. These include depression of serum sodium and cholesterol, instability of blood sugar with a tendency to hypoglycemia, and a tendency to hypotension common to both conditions. On the other hand, certain similarities exist between thermal and radiation burns. In the former condition Talbot et al⁽⁹⁰⁾ have shown that "corticosteroid" excretion is similar to that in conditions with hyperfunction of the adrenal cortex. Unfortunately,

no data was presented on the progressive changes found.

* The increased availability of tracer elements should give great impetus to studies of rates of turnover of numerous substances affected by radiation. These should give us a much more dynamic view of the problems involved.

In irradiated subjects, the increase in specific absorption at about 230 mμ in urinary "corticosteroid" extracts was the most consistently significant abnormality found by us. In the two instances where repeated analyses were made before death there was a terminal drop in the excretion of the substance responsible for this absorption. In one of these instances (Case 14) death was due to thermal burns. This would suggest an early hyperfunction of the adrenals followed by a terminal tendency to hypofunction.

As noted previously, time has not permitted adequate study of this phenomenon. The identity of the 230 mμ absorbing compound should be further investigated by study of its reactions, solubilities, and infra-red spectrum at least.

b. Porphyrin Studies.

Studies of urinary porphyrin excretion in industrial personnel appear to be of most importance as a screening reaction, since a large variety of conditions may cause elevated values. It is of greatest value in the study of personnel exposed to lead since marked elevations are generally found with significant exposure. Conversely, normal values may help rule out lead poisoning in some individuals with basophillic stippling of the red blood cells. It may also be of prognostic value in irradiated subjects.

The administration of lethal or near-lethal doses of total body radiation to dogs has been shown to result in a uniform depression of coproporphyrin excretion, followed usually by a terminal rise⁽⁷²⁾. It was postulated that the depression might be associated with the inhibition of erythropoiesis which also occurs, and the terminal rise might be due to the concomitant liver dysfunction.

Terminal urinary coproporphyrin excretion was elevated in the 3 patients studied. Repeated samples were available in only 2 of these. Concentration in the first 2 samples after radiation was low in the second

Los Alamos patient, who received an almost instantaneous exposure, but no control samples had been studied. In the other patient (Hospital Case #1 who received repeated exposure to 20-25 r total body x-ray) no early decrease in porphyrin excretion was noted.

Determination of the individual coproporphyrin isomer type in project personnel were carried out in over 100 cases. In all individuals with significant metal exposure, over 2/3 of the total porphyrin was the type III isomer. Control subjects, however, also had a predominance of the type III isomer in most cases, though the range of variation was much greater in these individuals. This is in contrast to the finding of Watson et al⁽¹⁰⁸⁾, who report a fairly consistent excess of the type I isomer in control subjects. The same method of analysis⁽⁸¹⁾ was used in both studies. The reason for this difference is unknown.*

Erythrocyte protoporphyrin studies appear to be of most value in subjects exposed to lead, or in individuals with iron-deficiency anemia. Neither radiation nor uranium exposure appeared to produce significantly elevated values.

c. Urorosein and other Urinary Pigments.

The most marked increases in excretion of urinary pigments were observed in the three patients who were ill at the time radiation therapy was given. Relatively slight changes only were found in the 2 previously healthy individuals who were accidentally exposed to lethal doses of radiation.

Several observations have been made which might conceivably be due to mechanisms similar to those relating to urorosein excretion,

(1) A terminal increase has been found in the excretion of kynurenic acid by dogs given a lethal exposure to total body x-radiation^(73,74).

Like indoleacetic acid, a precursor of urorosein, kynurenic acid is derived
*It may be that certain minimal exposures in our Chicago control group were actually significant. Further study of control subjects is in progress.

from tryptophane. (Little if any, indoleacetic acid appears to be excreted by dogs.) It seems, therefore, that a disturbance in tryptophane catabolism may be produced in both species by excessive radiation exposure.

(2) The inactivation of auxin (indoleacetic acid) by x-irradiation has been described by Skoog⁽¹⁰⁹⁾. He suggests that inhibition of growth of plants may be due to this factor.

(3) Macht^(110,111) has described an inhibition of *lupinus albus* growth on addition of serum from individuals exposed to even very small amounts of x-rays. These studies have not, to our knowledge been repeated. While the relationship here is especially tenuous, one wonders whether such an effect could conceivably be due to the presence of an altered indoleacetic acid or similar compounds in these sera.

(4) Stenstrom and Lohmann⁽¹¹²⁾ have reported the chemical alteration of tryptophan itself following exposure to x-irradiation.

Graham⁽¹¹³⁾ has reported the increased excretion of urine pigments (which he erroneously identified as coproporphyrin) in several irradiated patients. Watson and Layne⁽⁹¹⁾, on the other hand found no significant increase in uroporphyrin excretion in patients with carcinoma of the cervix following x-ray therapy (local).

d. Liver Function.

These tests will not be considered in detail here since they are discussed more fully elsewhere⁽⁷⁷⁾.

Though post-radiation changes in the albumin-globulin ratio have been reported by a number of investigators^(7,31), we have found no significant increases in serum cephalin cholesterol flocculation or in the serum colloidal gold reaction in our irradiated patients. In some, previous abnormalities actually disappeared for a while, at least. Terminal urinary urobilinogen increases were found in most of the patients. These

tests, like the urinary coproporphyrin excretion, seem to be of special value as "screening" reactions in the study of large groups of personnel. Further study is indicated by the finding of abnormal values.

Changes in the electrophoretic pattern of plasma from irradiated animals are discussed elsewhere in these volumes by Munis, et al⁽¹¹⁴⁾. Since these appear to be associated with the α_2 globulin^{and}, one might expect significant changes in the thymol turbidity test which is reported to be related to the γ globulin⁽¹¹⁵⁾. We have had little experience with this test in irradiated subjects. It was normal in Case I of the Los Alamos series the day before death.

e. White Blood Cell Chemistry.

These studies, which were given the highest priority in our group, are especially difficult to evaluate. Technical problems involving the quantitative isolation of intact white blood cells from venous blood, and their subsequent fractionation were especially time consuming. The isolation of the lipid-soluble fraction, for example, proved very troublesome because the usually accepted rules of solubility do not seem to apply when one is working with the mg or so of material present in our white blood cell sediments. The 95% ethyl alcohol extraction finally used is by no means the ideal solution to the problem.

Changes in the pentose concentration and in the ultraviolet absorption of some of the fractions in irradiated dogs are described elsewhere⁽⁷⁶⁾.

The significance of the increase found in the phosphorous concentration of the "adenosine triphosphate" fraction in at least one irradiated patient is uncertain. Unfortunately, these studies were quite incomplete in our human subjects.

It was originally intended to use the isolated white cells, especially as obtained by the gramicidin-lysolecithin method for metabolic studies. Time, however, did not permit this.

5. Summary and Conclusions.

From the above experimental studies and from a survey of the literature, the following conclusions have been drawn:

a. In no case were the biochemical alterations found in project personnel (exclusive of the Los Alamos group) the cause for alarm. Most highly abnormal scores were obtained in personnel who were ill because of an infection, or in those with a history of excessive chemical or metal exposure before joining the Plutonium Project.

b. The most striking biochemical changes found after excessive radiation exposure in humans were (1) an increase in urine "corticosteroid-like" substances in all of 5 individuals studied, (2) a prolonged increase in the "adenosine triphosphate" phosphorus in the peripheral white blood cells of a patient given total body x-ray for the treatment of a metastasizing parotid tumor, (3) pronounced terminal increases in urinary coproporphyrin excretion in 3 patients who died within a few weeks after heavy exposure to external radiations, and (4) increased excretion of "uroseal fraction" pigments in severely ill patients given large doses of total body x-irradiation. Other changes found included a shift to the left in the Arneth count, and an increase in erythrocyte sedimentation rate.

c. Determination of urinary uranium excretion yielded the best correlation with known uranium exposure in project personnel. The method devised for this purpose is sufficiently accurate down to levels of

0.0001 micrograms of uranium. This permits simple analysis on samples of only 0.05 ml of urine.

d. Determination of urinary catalase excretion yielded no information of value in project personnel exposed to uranium. (This test, however, does appear to be a sensitive index of acute uranium poisoning in rabbits.)

e. More fundamental information on the nature of very early radiation effects is urgently required from both diagnostic and therapeutic points of view. Such information might be obtained, for example, from (13,116,117) studies of physiologically important oxidation-reduction phenomena/and protein and enzyme effects.*

It is suggested that the white blood cells are ideally suited for many such studies in living subjects, since they combine the assets of radiosensitivity and accessibility to a greater degree than any other cellular elements in the body. Chemical studies of nucleic acids and various other phosphorus-containing compounds similar to those described herein might well be further pursued. The direct measurement of ultraviolet absorption by these cells by techniques similar to those of Caspersson⁽¹¹⁹⁾, and Mitchell⁽⁶⁾ also seems definitely indicated. Investigations might also include the study of metabolic properties such as respiration, glycolysis, and enzyme activities as well as other properties such as locomotion, opsonic index, and supravital staining.

f. The nature of relatively late and terminal radiation effects also requires further elucidation. The alterations found here in urinary excretion of corticosteroid-like substances, of coproporphyrin, and of uroporphyrin and other pigments require further investigation if their relation to radiation toxicity is to be better ascertained and understood.

A variety of non-specific late changes occur following excessive

* A recent report⁽¹¹⁸⁾ indicating that *Phycomyces Blakesleeanus* is sensitive to as little as 0.001 r seems to offer an especially fertile field for biochemical investigation.

exposure to penetrating radiations. These include, for example, signs of tissue breakdown and of terminal liver dysfunction. Allen has also shown recently⁽¹⁶⁾ that the late hemorrhagic tendency is due to the presence in blood of an increased concentration of a heparin-like substance, or possibly of heparin itself. Since these changes are of obvious clinical importance, their further study might be expected to yield information of therapeutic value at least.

The immuno-chemical study of protein destruction also appears to be a fertile field for investigation. The description of tissue specific proteinases in urine as well as of increased titer of blood antibodies to irradiated tissue proteins (though the latter is still controversial), are examples of the type of contribution which might come from these studies.

g. Where specific tests are available for the study of large groups of industrial personnel, they should obviously be given high priority. The urinary excretion of uranium and other metals are examples of such tests. Even these, however, should be supplemented where possible by tests of appropriate biochemical functions since the latter determine the clinical course of the overexposure.

In most instances, the use of non-specific "screening" tests appears to be the best available approach. The porphyrin, pigment and liver function studies done here fall in this category. In some instances they may become relatively specific, as for example in the study of urinary coproporphyrin in lead-exposed personnel. Those who react abnormally to these "screening" tests should be investigated more intensively.

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Table 1.

The Quantitative Basis of the Test Scores.

1. Urinary coproporphyrin

- 0 = 8 gamma percent or less
- 1 plus = 8-10 gamma percent
- 2 plus = 10-13 gamma percent
- 3 plus = 13-18 gamma percent
- 4 plus = more than 18 gamma percent

2. Red blood cell protoporphyrin

- 0 = 30 gamma percent or less
- 1 plus = 30-40 gamma percent
- 2 plus = 40-50 gamma percent
- 3 plus = 50-70 gamma percent
- 4 plus = more than 70 gamma percent

3. Cephalin cholesterol flocculation

0 to 4 plus flocculation as described by Hanger.

4. Colloidal gold test

(3 tube test as suggested by S. Gray)

- 0 = 3 tube total of 8 or less
- 1 plus = 3 tube total of 9 or 10
- 2 plus = 3 tube total of 11
- 3 plus = 3 tube total of 12 or 13
- 4 plus = 3 tube total of 14 or 15

5. Absorption at 400 mμ

- 0 = Evelyn reading of 100-60
- 1 plus = Evelyn reading of 60-50
- 2 plus = Evelyn reading of 50-30
- 3 plus = Evelyn reading of 30-20
- 4 plus = Evelyn reading of less than 20

6. 520 mμ absorption

- 0 = Evelyn reading of 100-75
- 1 plus = Evelyn reading of 75-60
- 2 plus = Evelyn reading of 60-40
- 3 plus = Evelyn reading of 40-25
- 4 plus = Evelyn reading of 25 or less

This type of scoring system was not applied to the urinary "corticosteroid-like" substances, catalase, or uranium.

Table 2.

White Blood Cell Phosphorus Fractions in a Patient Receiving 20-25 r
Total Body X-ray Almost Daily. (Total Skin Dose: 310 r)

	Control period (8 analyses)	Radiation Period (15 analyses)	Post-radiation period (6 analyses)
Total Phosphorus	2.18*	2.66	2.19
"Nucleoprotein" plus "Lipoid" P	1.46	1.80	1.50
Acid soluble P	0.76	0.76	0.64
"Adenosinetriphosphate" P	0.06	0.19	0.11
$\frac{\text{"ATP" P} \times 100}{\text{Acid soluble P}}$	7.9	24.2	17.0
"Hexosediphosphate" plus "phosphopyruvate" P	0.08	0.07	0.04
"Inorganic" P	0.20	0.17	0.13
Total WBC per cu. mm.	8300	7244	2500

* Average values are given in terms of micrograms phosphorus per million white blood cells. Total phosphorus is not equal to the sum of the nucleoprotein - lipoid phosphorus plus acid soluble P in these average values since one or the other of these determinations was not done in every instance.

Table 5.

Ultraviolet Absorption Ratios of "Corticosteroid" Fraction from Urine of Los Alamos Personnel and Control Subjects.

Case	Exposure	Date	$\frac{a}{220/240 \text{ m}\mu}$	$\frac{b}{240/260 \text{ m}\mu}$	a/b
1.	Lethal Radiation	3-21-45	1.45	3.6	0.40
2.	Lethal Radiation	5-21/22-46	1.28	1.36	0.94
		5-22-46	1.47	1.17	1.25
		5-26/27-46	0.93	1.62	0.57
		5-27/28-46	1.09	1.40	0.78
		5-28/29-46	1.70	1.18	1.44
3.	"Severe" Radiation	5-22/23-46	1.40	1.32	1.06
		5-26/27-46	1.40	1.75	0.80
		5-27/28-46	1.07	1.95	0.55
		5-30/31-46	1.43	1.58	0.90
		5-31/6-1-46	1.91	1.52	1.25
		6-9-46	1.62	1.06	1.53
		6-12/13-46	1.49	1.15	1.30
4.	"Severe" Radiation	5-21-46	1.40	1.53	0.92
		5-21/22-46	0.78	2.86	0.27
		5-27/28-46	1.31	1.72	0.76
		5-28/29-46	1.31	1.72	0.76
		5-29/30-46	1.29	1.73	0.75
		6-21-46	1.40	1.50	0.93
		6-27-46	1.37	1.67	0.82
5.	"Severe" Radiation	5-27/28-46	0.92	2.63	0.35
		6-9-46	1.43	1.59	0.90
		6-11/12-46	1.32	1.87	0.71
6.	"Light" Radiation	5-21/22-46	1.88	0.85	2.21
7.	"Light" Radiation	5-21/22-46	1.88	1.08	1.74
8.	"Light" Radiation	5-21/22-46	1.97	1.07	1.84
9.	"Light" Radiation	5-21/22-46	1.72	0.98	1.76
10.	Control		1.69	0.97	1.74
11.	Control		1.55	1.08	1.44
12.	Controls		1.60	0.89	1.80
13.	Lethal Thermal Burn	6-5-46	1.15	1.77	0.65
		6-8-46	1.38	1.33	1.04

Table 4.

Urinary Coproporphyrin Excretion
in a Case of Acute Lethal Over-
exposure to External Radiation.
(Case 2)

Date	Urine vol. (ml.)	Coproporphyrin Excretion	
		micrograms %	micrograms/day
5-21/22	310	1.8	6
5-22 (12 hrs.)	500	2.4	24
5-26/27	990	18.8	186
5-27/28	1770	25.5	451
5-28/29	466	25.1	117

Table 5.

Correlation of Uranium Exposure and Urinary
Uranium Concentration in Project Personnel
(Iowa State College Project)

<u>U</u> <u>Exposure</u>	<u>No. of</u> <u>Individuals</u>	<u>No. of</u> <u>Samples</u>	<u>Percent of samples containing</u> <u>micrograms of U per liter.</u>				
			<u>0-10</u>	<u>10-20</u>	<u>20-40</u>	<u>40-80</u>	<u>80-200</u>
Incidental	6	6	100	0	0	0	0
Light/	11	14	35	29	35	0	0
Moderate	20	26	0	23	27	31	19
Heavy	11	21	0	5	14	43	38

Table 6.

Urinary Catalase Activity in Control Subjects and in
Project Personnel with "Heaviest" Exposure to
Uranium.

Uranium Exposure	Blank	# Analyses	# Individuals	Range thiosulfate Difference (ml)	Average Difference (ml)
None or Incidental	#1 (urine)	35	20	0.00-0.12	0.05
	#2 (urine)	48	23	0.00-0.18	0.05
	H ₂ O	48	23	0.02-0.24	0.10
"Moderate" or "Heavy"	#1 (urine)	34	19	0.00-0.16	0.06
	#2 (urine)	55	26	0.00-0.31	0.05
	H ₂ O	54	26	0.01-0.31	0.12

Table 7.

Total Urinary Coproporphyrin Concentration
in Different Groups of Subjects. Elevated Scores.

Exposure	# Tests	%3-4 + Scores
Controls (chiefly medical students)	50	10
Polycythemia and leukemia patients	121	5
"Chemists" (Chicago)	125	21
TO ₂ F ₂	27	70
TO ₂	185	32
TF ₄ (Ames)	112	21
"Chemists" (Ames)	69	38

Table 8.

A Comparison of A.M. and P.M. Urinary Coproporphyrin and Pigment ("Urorosein Fraction") Concentrations.

Coproporphyrin (γ%)			Urorosein Fraction 400 mμ		Unoxidized 520 mμ		Total Score	
	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.	A.M.	P.M.
1.	9.5 (1+)	25.5 (4+)	0	2+	0	1+	1	7
2.	15.4 (3+)	24.4 (4+)	2+	2+	0	1+	5	7
3.	14.8 (3+)	15.3 (3+)	1+	2+	1+	2+	5	7
4.	13.3 (3+)	3.7 (0) *	0	2+	0	1+	3	3
	23.0 (4+)	17.0 (4+)	2+	2+	2+	2+	8	8
5.	9.2 (1+)	13.1 (3+)	0	2+	2+	2+	3	7
	7.8 (0)	8.7 (1+)	0	1+	0	1+	0	3
6.	3.8 (0)	8.5 (1+)	0	1+	0	2+	0	4
7.	7.9 (0)	6.2 (0)	1+	0	2+	1+	3	1
8.	30.4 (4)	21.2 (4)	3+	2+	2+	2+	9	8
9.	6.6 (0)	9.2 (1)	--	--	--	--	--	--
10.	3.7 (0)	8.5 (1)	--	--	--	--	--	--
11.	1.7 (0)	7.0 (0)	--	--	--	--	--	--

* The only one of 10 samples from this individual which was less than 2+.

Table C.

A Study of the Consistency of Urinary Coproporphyrin
Concentration in Project Personnel.

Subject	Number of Analyses	Period (weeks)	Average micrograms %	Standard deviation micrograms %
1.	5	21	34	7.5
2.	8	22	18	4.6
3.	19	4	18	5.3
4.	10	2	18	2.9
5.	22	4	15	6.7
6.	22	4	15	5.5
7.	21	4	15	4.6
8.	17	4	14	5.8
9.	9	1½	13	4.3
10.	19	4	12	4.1
11.	5	15	12	1.3
12.	19	4	11	4.8
13.	7	27	11	3.6
14.	14	4	11	4.4
15.	8	23	10	4.6
16.	6	25	8	4.3
17.	6	12	6	4.0
18.	6	30	5	2.2
19.	6	35	5	2.6
20.	5	32	5	1.4

Same range of values found in 15 other employees of the Manhattan Project
on whose urine at least 5 different coproporphyrin analyses were done.

Predominant exposure was to metals and chemicals in all instances.

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Table 10.

Porphyrin Studies of 5 Individuals Exposed to Varying Amounts of Lead (Argonne Laboratory)

Case	Date	RBC Protoporphyrin* Micrograms %	Urine Coproporphyrin**	
			Micrograms %	Micrograms/day
1.	7-11	281	16	218
	7-14	---	22	314
	7-31	2,280	---	---
	8-3	---	48	588
	8-20	210	3	35
2.	7-11	31	16	223
	7-14	---	24	177
	7-31	54	---	---
	8-3	---	8	50
3.	7-11	39	9	115
	7-14	---	21	149
4.	7-11	92	10	286
	7-31	90	---	---
	8-3	---	15	142
	8-24	92	---	---
5.	7-11	94	7	89
	7-14	---	5	72

* Upper limit of normal, about 40 micrograms per cent.

** Upper limit of normal about 10 micrograms per cent and 100 micrograms per day.

Two urinary lead analyses on 7-11 and 7-14, case 1, showed values of 500 and 100 micrograms lead per liter. One to 7 micrograms uranium per liter in urine of above 5 cases on 7-11. Six and 7 mg. urobilinogen per day present in urines of cases 3 and 2 on 7-11. Others normal.

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Table 11.

Urinary "Urorosein" Scores in Different Project Groups.

Predominant Exposure	# Subjects	Unoxidized Fraction		Oxidized Fraction	
		400 mu %2-4 +	520 mu %2-4 +	400 mu %2-4 +	520 mu %2-4 +
Controls	33	6	3	6	6
Mixed Chemical and Metal; (Slight)	57	16	9	28	32
Chemicals; Radia- tion	41	20	20	48	37
Uranium	80	14	17	24	29
Other Metals	51	16	17	26	40

Table 12.

Absorption Bands (Spectroscopic) in
"Urorosein" Fraction, Unoxidized

Predominant Exposure	# Subjects	490-495 mμ		510 mμ		543 mμ	
		# analyses	% +	# analyses	% +	# analyses	% +
Controls	32	32	0	33	3	33	6
Mixed Chemical and Metal (Slight)	56	62	0	65	2	65	12
Chemicals: Radiation	55	78	0	84	1	84	33
Uranium	37	54	3	54	8	54	8
Other Metals	38	43	8	44	0	43	3
Unclassified	52	62	2	62	2	62	10

Table 13.
Absorption Bands (Spectroscopic)
in "Urorosein" Fraction, Oxidized.

Predominant Exposure	# Subjects	490-495 mμ		510 mμ		543 mμ	
		# analyses	% +	# analyses	% +	# analyses	% +
Controls	32	32	18	33	6	33	39
Mixed Chemical and Metal (Slight)	51	57	0	60	4	60	69
Chemicals; Radiation	61	74	2	90	8	90	90
Uranium	37	54	4	54	22	54	57
Other Metals	38	39	16	42	0	45	79
Unclassified	52	62	2	62	6	62	60

Table 14.

Studies of Liver Function in Various Groups of
Project Personnel.

		Predominant Exposure				
		Controls	Uranium	Other Metals	Radiation	Chemicals
Number of Individuals, Total		38	51	37	12	78
Cephalin Cholesterol;	Number + * Tests	4	6	6	0	12
	% of + Tests	10	12	18	0	15
	Number 3 - 4 +	0	2	2	0	2
	% 3 - 4 +	0	4	6	0	2
Colloidal Gold:	Number + Tests	1	3	4	1	10
	% + Tests	3	6	12	9	13
	Number 3 - 4 +	0	2	4	0	4
	% 3 - 4 +	0	4	12	0	5
Urobilinogen:	No. Tests Done	18	28	30	7	47
	Number + Tests	5	0	9	0	11
	% + Tests	16	0	25	0	21
	Number 3 - 4 +	2**	0	5	0	5
	% 3 - 4 +	11	0	11	0	10

* 2-3+ scores.

** History of recent albuminuria and hematuria in one case. 2+ cephalin cholesterol also present in other case.

Table 15.

Correlation of Liver Function
Studies in Project Personnel.

	3 to 4 + Scores					
	C.G. (31)		C.C. (13)		U.U. (16)*	
	#	%	#	%	#	%
C.G. (2-4 +)	-	-	5	42	1	8
C.C. (2-4 +)	11	36	-	-	4	29
U.U. (2-4 +)**	2	13	2	50	-	-
Copro. (2-4 +)	11	38	7	58	11	75
R.B.C. Proto. (2-4 +)	7	27	3	27	2	14

C.G. - Colloidal Gold

C.C. - Cephalin-cholesterol flocculation

U.U. - Urine Urobilinogen

Not all tests were done in every instance. The % values are therefore calculated only for those instances where both tests being correlated were performed.

* over 1 mg. per cent. Watson's modified semiquantitative procedure used.

** analyses done in less than half the cases.

Table 16.

Composite Scores, Control Medical Students.

Subject	C.C.	C.G.	R.P.	U.C.	400 mμ	520 mμ	510 band	Total Score
1	0	0	1	0	0	0	0	1
2	1	0	0	0	0	0	0	1
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6*	0	0	3	1	1	0	0	5
7	1	1	0	0	0	0	0	2
8	0	1	0	0	0	0	0	1
9	1	1	1	0	0	0	0	3
10	1	0	0	0	0	0	0	1

* 8 grams sulfathiazole 4 days previously.

C.C. - Cephalin cholesterol flocculation

C.G. - Colloidal gold

R.P. - Red cell protoporphyrin

U.C. - Urine coproporphyrin

Table 17.

Composite Scores, Exposed Project

Personnel.

	C.C.	C.G.	R.P.	U.C.	400 mμ	520 mμ	510 band	Total Score
1	1	2	1	3	2	2	+	12
2	4	1	1	2	2	3	+	14
3	1	2	2	0	1	1	+	8
4	3	1	0	0	0	0	+	5
5	1	-	0	4	3	4	+	13
6	1	3	2	2	1	1	0	10
7	1	1	4	0	2	0	0	8
8	1	1	0	1	2	2	0	7
9	0	3	1	0	1	1	0	6
10	1	0	2	1	3	3	0	10
11	4	4	-	1	4	3	0	16

C.C. - Cephalin Cholesterol flocculation.

C.G. - Colloidal gold.

R.P. - Red cell protoporphyrin.

U.C. - Urine coproporphyrin.

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