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QUARTERLY TECHNICAL REPORT

April 1 thru June 30, 1954

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QUARTERLY TECHNICAL REPORT

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It should be noted that the Quarterly Technical Reports of The University of Rochester Atomic Energy Project do not attempt to describe progress in all of the research programs but only in those in which some significant results have been achieved but which are not sufficiently complete to be written up as final reports.

Submitted by: Henry A. Blair
Director

Date of Report: 8/26/54

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A COLORIMETRIC METHOD FOR THE DETERMINATION OF Zr IN $ZrOCl_2$

by
H. B. Wilson

ABSTRACT

A satisfactory colorimetric method for the determination of Zr in $ZrOCl_2$ is described. The method is based on that of Hayes and Jones (1). The dye used was an Eastman Organic Chemical labeled "p-dimethylaminoazophenylarsinic acid". Satisfactory results were not obtained when the recommended p-dimethylaminoazophenylarsonic acid was used.

* * * * *

Background: When a study of the chronic inhalation toxicity of $ZrOCl_2$ was undertaken, an accurate colorimetric method for small amounts of Zr was needed. Hayes and Jones had determined zirconium in steel by a colorimetric method using p-dimethylaminoazophenylarsonic acid. A few grams of an Eastman Organic Chemical labeled "p-dimethylaminoazophenylarsinic acid" were available. A satisfactory method, using this dye and a modification of the procedure of Hayes and Jones, has been developed.

Standard $ZrOCl_2$ Solution: The Zr content of a stock solution of $ZrOCl_2$ (prepared from pure $ZrCl_4$) is found gravimetrically by addition of NH_4OH to a diluted aliquot, and ignition of the precipitated hydroxide to the oxide. To an aliquot of this stock solution are added concentrated HCl and water sufficient to make the normality approximately 6. This solution is boiled about 1 minute, cooled and made up with aq. to volume in a volumetric flask. The final HCl normality should be approximately 1.2. This is the standard solution.

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1. Hayes, Walter G. and Jones, Edward W., Determination of Zirconium in Steel, Ind. Eng. Chem. Anal. Ed. 13, 603 (1941).

Reagents: Dye solution. 1.00 g "p-dimethylaminoazophenylarsinic" acid in 40 ml concentrated HCl, made up to a 1 liter with ethyl alcohol. HCl wash, 10 ml concentrated HCl in 1 liter aq. Ammonium hydroxide wash, 333 ml NH_4OH (0.90 spgr.) to 1 liter.

Method: To the sample add concentrated HCl and dilute to 6 N with aq. Bring to a boil, cool and dilute to volume in a volumetric flask. The volume of 6 N HCl should be 20% of the flask volume so that the final normality is approximately 1.2. If necessary, there should be a further dilution with 1.2 N HCl so that an aliquot containing 0.1 to 0.3 mg Zr may be taken for analysis.

Add an aliquot, from the solution to be analyzed, to sufficient 1.2 N HCl (containing a pulped 9 cm filter paper) to make 70 ml. Bring to a full boil. Add 30 ml of the dye solution. Let stand about 1 hour. Filter through two 12.5 cm No. 40 Whatman filter papers and wash with the HCl wash water until the filtrate is practically colorless. Fifteen to 20 washings will be required. Remove the dye combined with Zr by washing with several portions of the NH_4OH wash solution, into a 100 ml volumetric flask. Find the Klett-Summerson scale reading using the blue filter. From the scale reading find milligrams of Zr by reference to a standard curve plotted from 3 standards analyzed simultaneously with the sample. A typical standard curve is shown in Figure 1.

To test the accuracy of the method for filter-paper-dust-sample analysis, 2 ml of a $ZrOCl_2$ solution (900 ppm Zr) were added, respectively, to each of three 3 cm disks of Whatman No. 41 filter paper in a small beaker. Each was evaporated to dryness at a temperature below boiling; 20 ml of 6 N HCl were added and the beakers' contents brought to a boil. Each solution was transferred to a 100 ml flask and made up to volume with aq. A 10 ml aliquot of

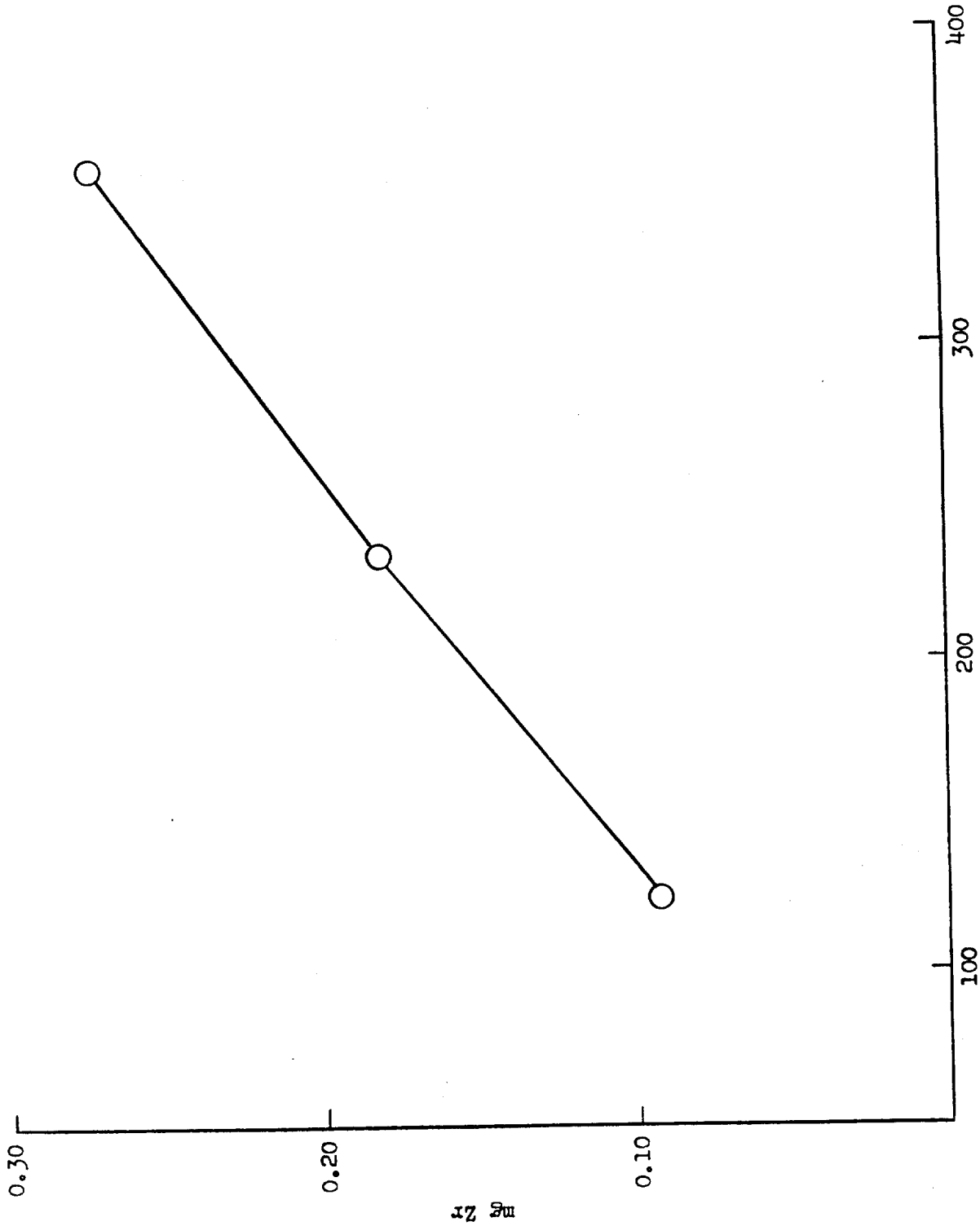

Figure 1. Standard Zr Curve

each was analyzed colorimetrically. Results are listed in Table 1. Also tabulated are comparative results of analyses of a dilution of a solution prepared from $ZrCl_4$ and of a solution of Mathesons hydrated $ZrOCl_2$.

Table 1

	mg.Zr	
	Gravimetric	Colorimetric
Filter paper sample No. 1	0.180	0.182
Filter paper sample No. 2	0.180	0.183
Filter paper sample No. 3	0.180	0.182
$ZrOCl_2$ sol. from $ZrCl_4$	0.114	0.114
Mathesons hydrated $ZrOCl_2$	0.127	0.128

Regarding details of the method, 1) it is of course not necessary to use Whatman filter paper, and the filter paper pulp may be superfluous. It does, however, minimize creeping. 2) Smaller volumes of solutions may give satisfactory results. 3) The importance of boiling with 6 N HCl and of holding the normality at 1.2 thereafter is illustrated by the following experiment: 10 ml of a $ZrOCl_2$ solution (containing 90 ppm Zr in 0.06 N HCl) were treated respectively as follows: A. 10 ml conc. HCl were added, the mixture boiled a few seconds, cooled and transferred to a 100 ml flask. B. Same as A except that the mixture was not heated. C. 60 ml of distilled water were added and then 10 ml conc. HCl in a 100 ml flask. Each was made up to volume and a 20 ml aliquot of each taken for test. Results are shown in Table 2.

Table 2

	MG Zr	
	Gravimetric	Colorimetric
A	0.180	0.182
B	0.180	0.166
C	0.180	0.154

Aging in 1.2 N HCl gives the same result as heating in 6 N HCl but three or four days may be required.

The "p-dimethylaminoazophenylarsinic acid", an Eastman Organic Chemical, listed as such in older Distillation Products catalogues, had been on our shelves for several years. However, correspondence with a representative of Distillation Products has elicited the information that it was misnamed and, in fact, was the same compound as the p(p-dimethylaminophenylazo) benzenearsonic acid that is listed and sold today. But neither this latter Eastman chemical nor Mathesons p-dimethylaminoazephenylarsonic acid have given satisfactory results. The difference between the two Eastman chemicals is shown by the following experiment. In each of three tests, 50 ml of a $ZrOCl_2$ solution containing 0.228 mg Zr in 1.2 N HCl were brought to a boil in a 250 ml beaker, 15 ml of dye solution added, the beaker covered with a watch glass and contents boiled 1 minute, removed from the hot plate, let stand about an hour and filtered through three fine texture filter papers. Nearly 40 washings with HCl wash solution (1 part HCl in 100 parts aq.) followed. A 100 ml volumetric flask was then placed under the funnel and the dye removed by repeated washing with NH_4OH solution (333 ml to 1 L) until the flask was nearly full. This is essentially the procedure in detail recommended by Hayes and Jones. The NH_4OH treatment was now repeated with a second and with a third flask. Each was made up to volume and Klett-Summerson scale readings recorded. Results are shown in Table 3.

Table 3

Dye	Klett Scale Readings Flask Number 1	2	3	Final Appearance of Filter Papers
A Eastman "arsinic"	287	1	0	colorless
B Eastman "arsonic"	290	61	26	very yellow
C* Eastman "arsonic"	302	56	30	very yellow

* C was the same as B except that the solution contained 100 mg of ferrous iron.

AN ELECTRONIC RELAY

by
Thomas P. Davis

ABSTRACT

An electronically operated relay has been developed which, while primarily designed for use with sensitive mercury thermoregulators, may be employed whenever a large power boost is required of delicate or high resistance contacts. Use of a vacuum triode rather than a thyratron permits high grid circuit resistances with resultant low control current. Reliable operation has been secured with about 0.8 volt across the open control contacts, yielding a current of 0.4 microamps through the contacts on closure.

****

Among the several excellent, highly sensitive mercury thermoregulators commercially available, almost all must be used with some auxiliary relay to enable the delicate contacts to handle the high power required by heating or cooling equipment. The simple electronic relay described below is designed primarily for such service, although it incorporates sufficient flexibility to recommend its use whenever considerable power boost is required of delicate or high resistance contacts.

While the relay is quite versatile, its principal advantage is simplicity of design and construction. The basic circuit, shown in figure 1, is an ac operated vacuum triode with grid operating potentials obtained from the 6.3 volt secondary of the filament transformer. The dotted ends of the transformer windings indicate like instantaneous polarities.

In operation, the bias control is advanced sufficiently far to close the plate relay with the thermostat contacts open; when these latter close, the negative voltage impressed on the grid is sufficient to reduce plate

current nearly to zero, permitting the relay to drop out. The electrolytic capacitor across the relay serves the dual function of preventing ac chatter, and making the relay sluggish enough to avoid erratic clatter when the thermoregulator is just making or breaking contact. Although this circuit operated satisfactorily with a 6J5, the two sections of a 6SN7 in parallel give a more adequate power reserve for the particular plate relay selected.

While this basic circuit is quite satisfactory for many applications, several minor additions will provide much greater flexibility of operation. This refined circuit, shown in figure 2, provides a differential adjustment, and choice of internal or external power to the controlled load. A push button test switch across the thermostat contacts is also added to facilitate adjustment of the unit.

In use, the bias control is adjusted as outlined above, then the differential control advanced until the decrease in plate current caused by closure of the control contacts (or test switch) is just sufficient to permit the plate relay to drop out. In this way, the minimum possible voltage is placed across the thermostat contacts consistent with reliable operation; in the unit pictured in figure 3, this condition is secured with approximately 0.8 volts rms across a Princo "Magna-set" thermoregulator, resulting in a control current of about 0.4 microamperes. Alternatively, with the differential control returned to provide maximum negative voltage, the unit operates satisfactorily with 6 megohms in series with the thermostat contacts. By using a higher grid resistance than 2 megohms, this latter value may be increased, if necessary. It should be noted that the use of a vacuum triode rather than a thyratron permits these high grid circuit resistances, with resultant low control current.

The DPDT "Internal-External" switch permits selection of power for the controlled load from an internal connection to the 117 volt ac line or from a pair of panel mounted binding posts. By placing a jumper between these binding posts, the unit can be used in applications requiring normally open or normally closed unpowered contacts completely isolated from the ac line.

As indicated in figures 1 and 2 it is important to connect the return side of the power line to ac ground in order to prevent placing the thermostat contacts at approximately full line voltage above ground. Since, however, even with this precaution, neither control contact is strictly at ground potential, a one-to-one isolation transformer should be used in applications where such grounding is essential.

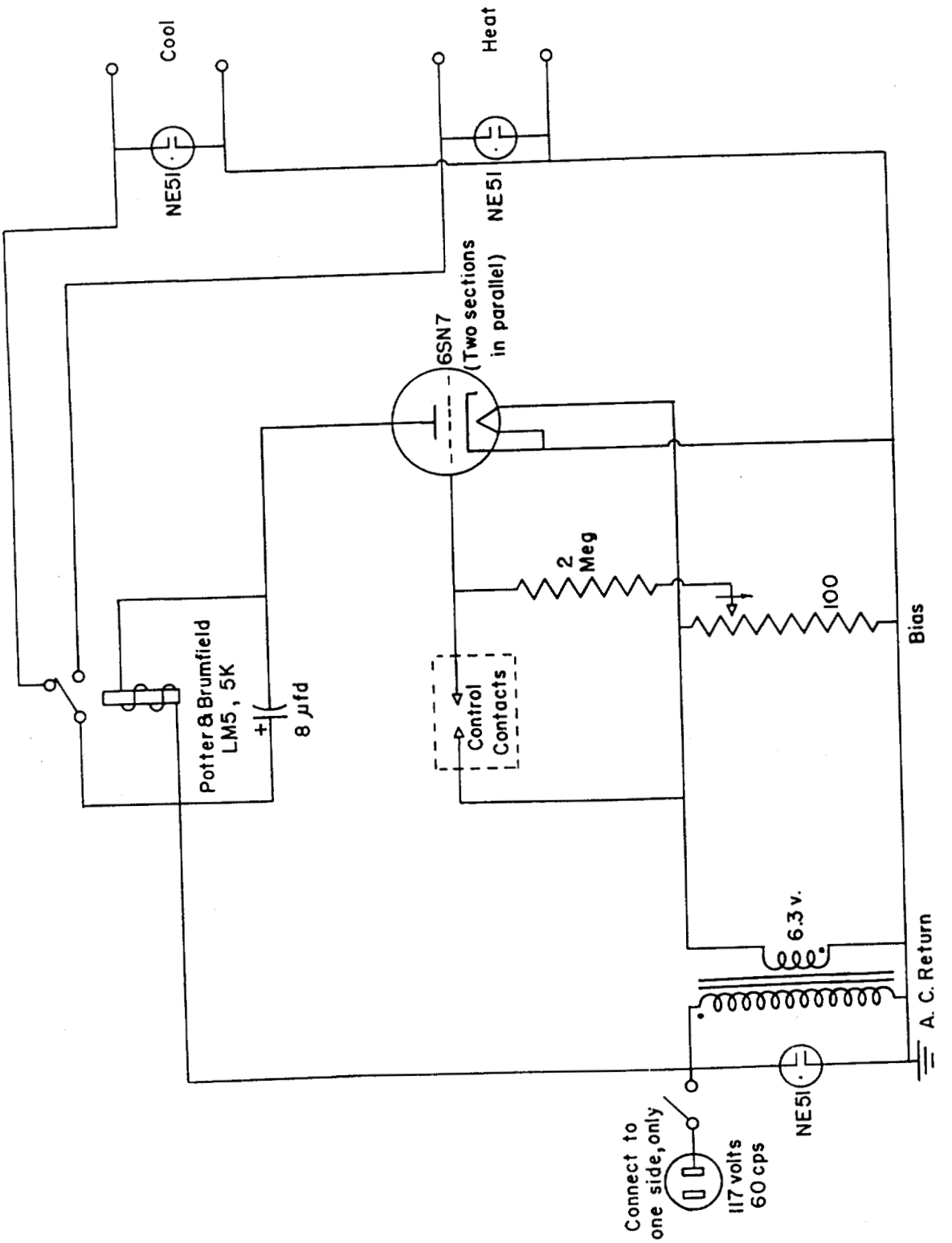

Figure 1

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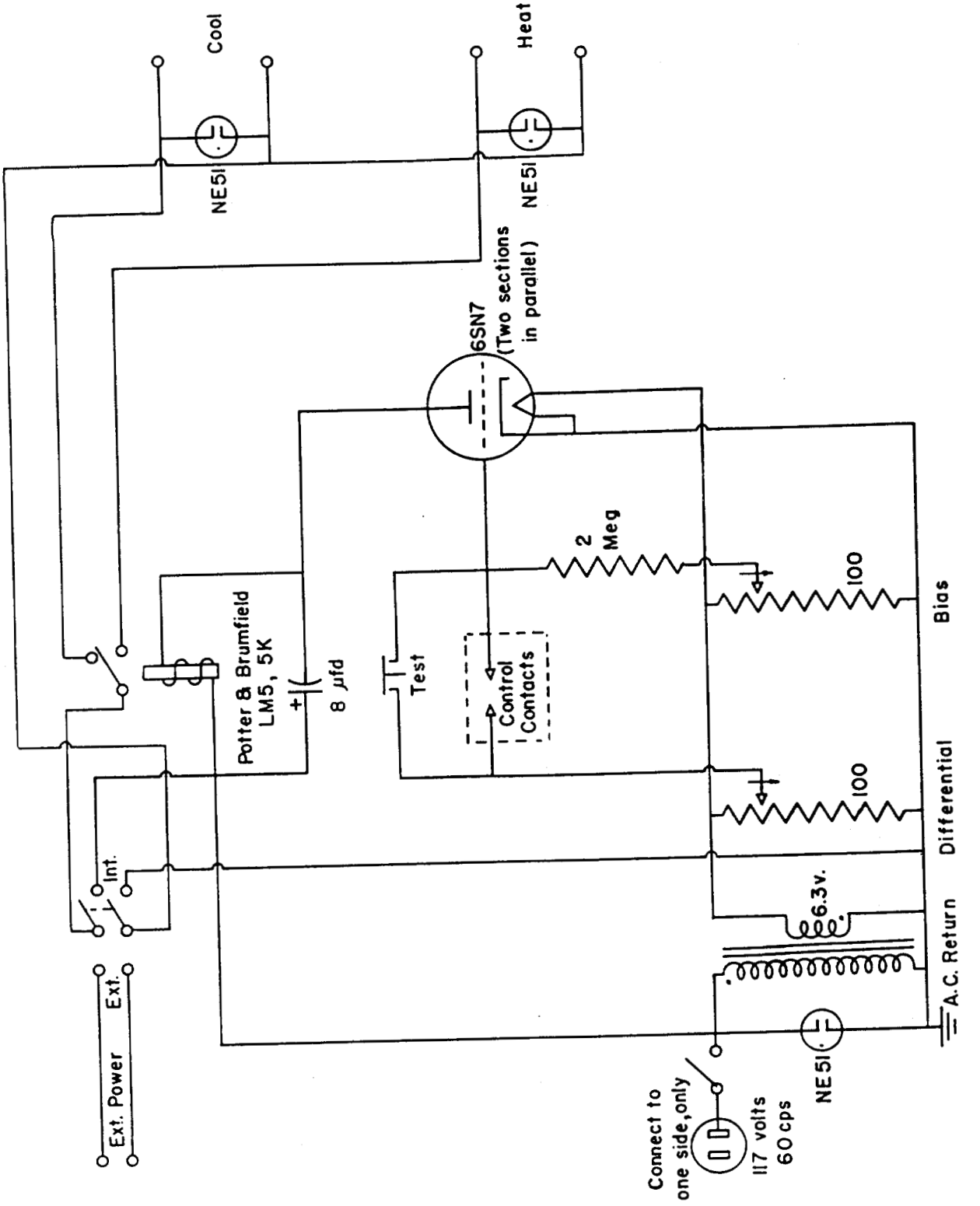

Figure 2

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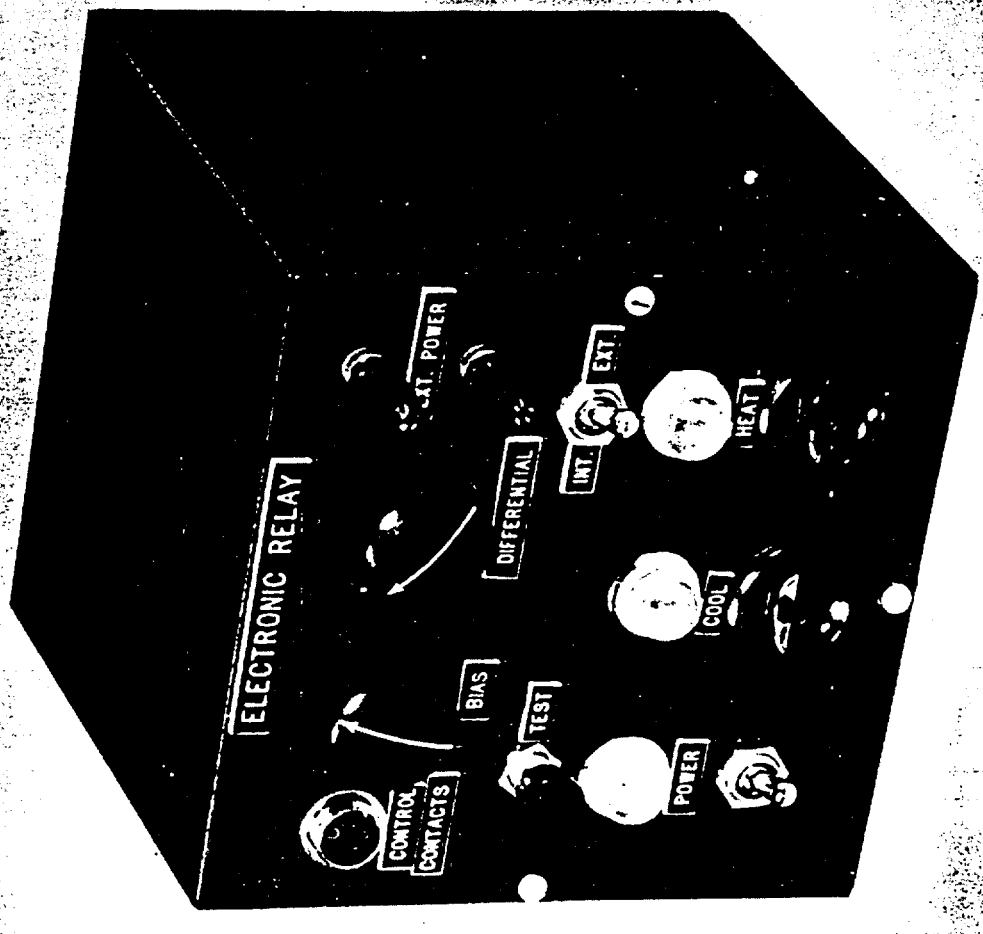
ELECTRONIC RELAY
CONTROL CONTACTS
BIAS
TEST
POWER
DIFFERENTIAL
INT.
EXT.
COOL
HEAT

Figure 3

AN IMPROVED AEROSOL GENERATOR

by
K. E. Lauterbach, A. D. Hayes, and M. A. Coelho

ABSTRACT

An improved aerosolizer has been constructed for production of heterogeneous aerosols from either suspensions of ground insoluble materials or solutions of soluble compounds. The aerosolizer operates on the conventional aspiration principle, but uses the surface tension of the solution to draw liquid up to and into the air jet. Therefore, since no capillary is used for liquid flow, plugging difficulties have been eliminated.

The system is being tested to determine performance characteristics with solutions of 10.0, 1.0, and 0.1% NaCl operating the jet above and below the liquid surface. Preliminary tests indicate that the generator will produce uniform aerosol concentrations for periods up to six hours. Particle size values of the aerosol have been found to be reproducible at each of the tested conditions. Further information will be given in a later report.

* * * * *

An improved aerosol generator has been constructed for use in radioactive particulate studies. Because of the complexity of the required dry-box procedures, it was essential that complete data on performance of the generator be obtained before its use with radioactive compounds. Preliminary tests of the new generator, which operates on the aspiration principle, indicate that it is nearly free of operational difficulties. Tests will be completed to determine mass concentration and mass median diameter of the test aerosol produced from solutions of sodium chloride at concentrations of 0.1%, 1.0%, and 10%. On the basis of these studies it may be possible to establish the relationship of particle size and concentration to solute concentration in the

aspirated solution.

The aerosol unit consists of a generator flask and reservoir, connected as shown in Figure 1. Solution flows from the reservoir through a section of capillary into the generator. A constant liquid level is maintained by the overflow tube which in turn is connected to an air-lift pump to return solution to the reservoir. During operation of the jet, 150 ml of solution is continuously recycled at the rate of 5 to 10 cc/min. in order to minimize the change in sodium chloride concentration due to evaporation losses. Data showing the increase in concentration of the solution as a result of water evaporation by the air jet have been obtained and will be presented in a later report.

The jet tube is made from 1/2 inch diameter Lucite rod to the dimensions shown in Figure 2. The air jet, formed by a .0125 inch hole near the base of the tube, is positioned with respect to the surface of the solution by adjustment of the standard taper collar. With this arrangement the jet can be removed and then replaced in the same position in the flask.

It is interesting to note that in this generator the air jet is operated without an opposing capillary for liquid flow, performing satisfactorily either submerged or not submerged. In the latter case, if the air jet is positioned from 1/32 to 1/8 inch above the surface of the solution, surface tension of the solution pulls liquid up to and around the jet opening. The high velocity air column picks up this liquid to form a dense spray which impinges against the walls of the container. The aerosol formed is removed through a vertical side-arm on the flask.

Probably the most troublesome feature of any aspirator system is the tendency of the air jet to plug due to lint or other particulate material in the air supply. Considerable care was devoted therefore to assembly of an air cleaning train which would eliminate solid contaminants in the jet air.

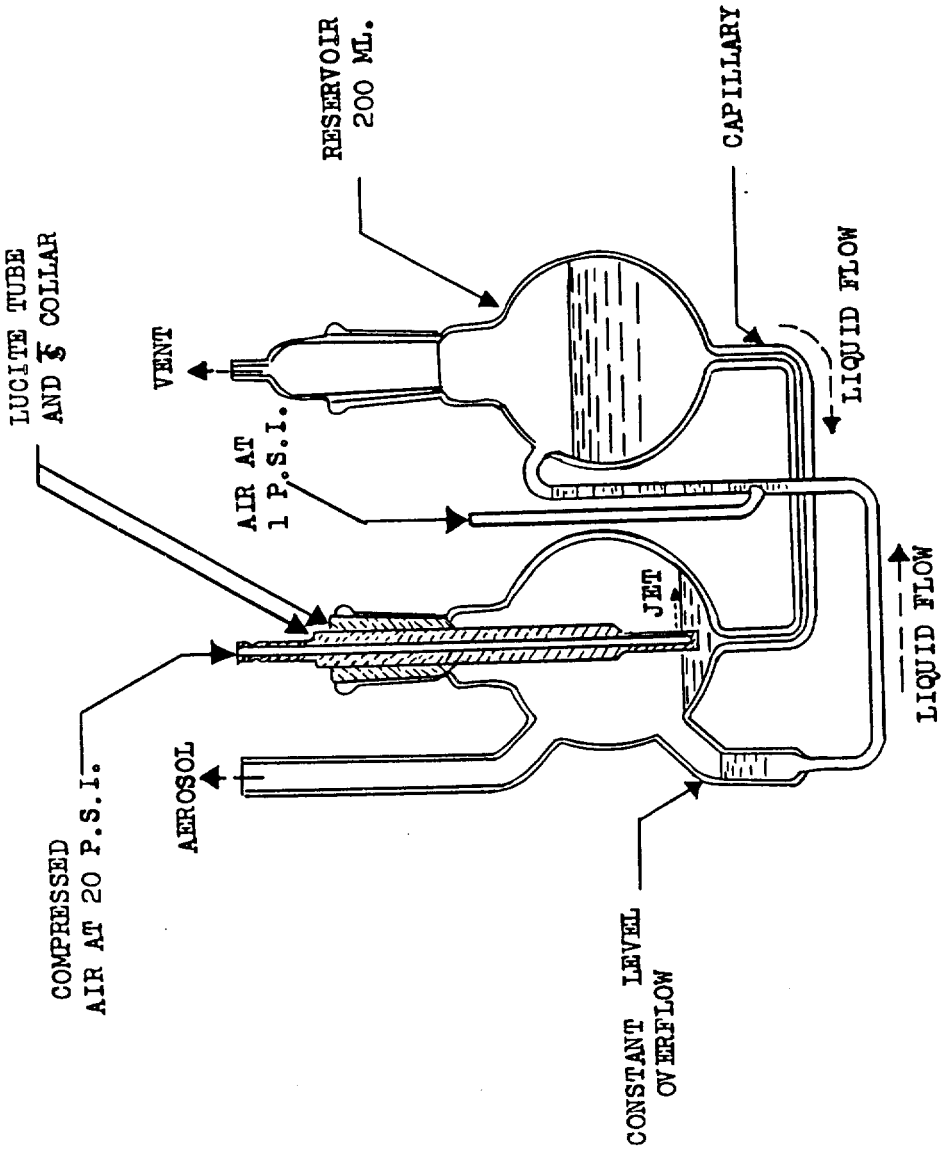

Figure 1. Diagram of the Aerosol Generator

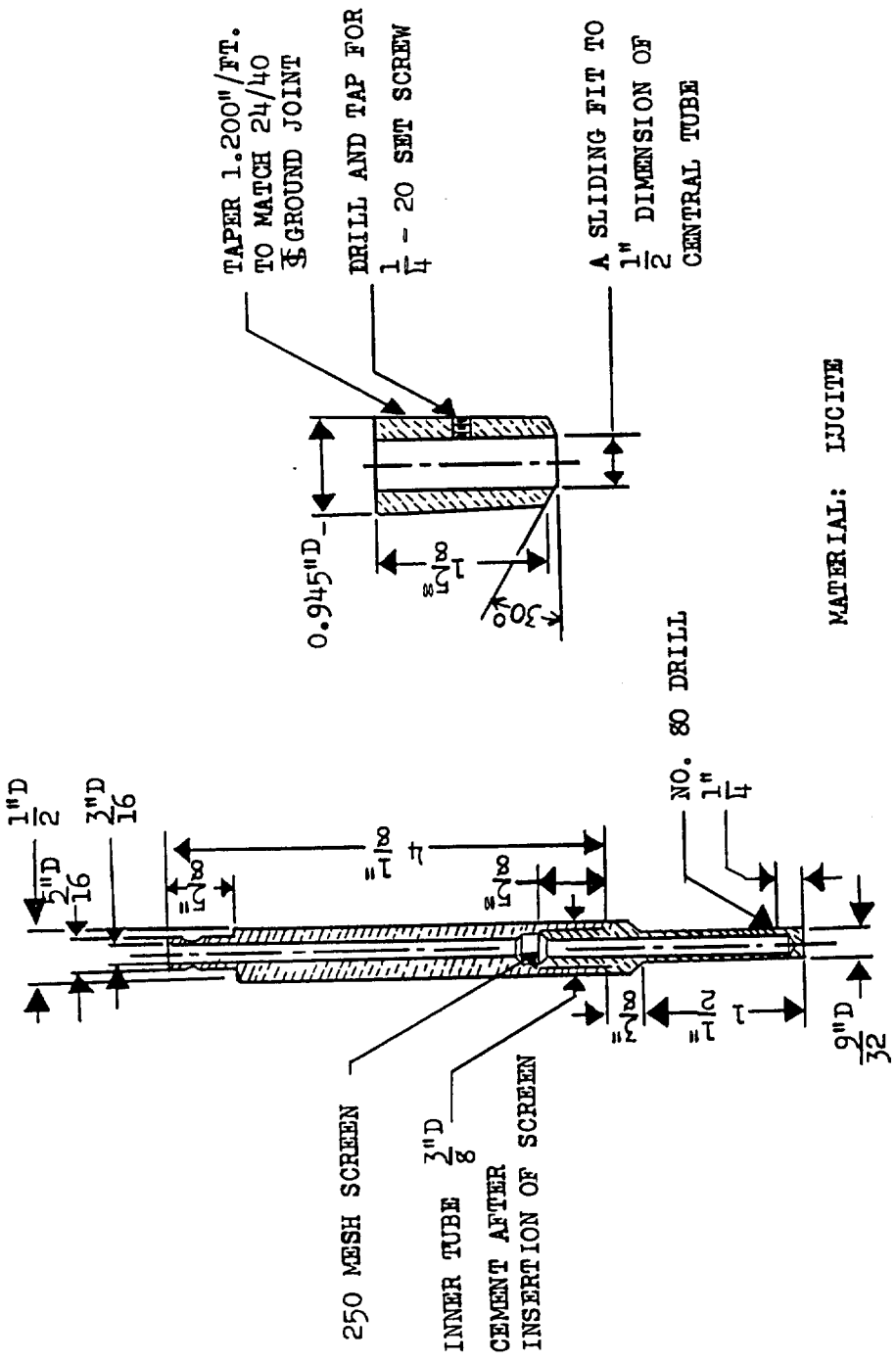

Figure 2: Diagram of the Jet Tube.

Although various traps and filters were tried, it was not until a molecular filter in a special holder was inserted in the air line that this difficulty was eliminated. The system finally adopted consisted of the following series of equipment: 1) pressure regulator, 2) glass wool filter, 3) humidifier, 4) molecular filter, 5) pressure gauge, 6) jet. All connections after the molecular filter including the pressure gauge connection were either glass or Tygon tubing. A further precaution against any large particles which might be introduced from this tubing was the use of a 250 mesh stainless steel screen within the jet tube itself. This system has performed entirely satisfactorily in all of the tests so far conducted. The generator has been tested for periods up to six hours with sodium chloride solutions of various concentrations and shows a very uniform production of aerosol. Molecular filter samples have been taken at 30 minute intervals during the six hour test and analyzed for sodium by the flame photometer. The results showed that individual variations of all samples were less than $\pm 5\%$ from the mean at each concentration.

Other tests will be conducted with this generator to confirm these results and also to establish the particle size of the aerosol produced from various concentrations of sodium chloride solution.

Abstract of Paper Presented at
the 38th Annual Federation Meeting
Atlantic City, New Jersey, April 11-14, 1954

ROLE OF ELECTROLYTES AND STARVATION IN ALTERING APPARENT RADIOSENSITIVITY OF BAKERS' YEAST

by
William J. Bair and J. N. Stannard

Ordinarily respiration and fermentation in yeast and other microorganisms are not markedly altered by radiation doses sufficient to prevent colony formation. However, experiments now completed show that, when measurements are made in a buffer system free of potassium and phosphate (other conditions being the same), aerobic and anaerobic CO_2 production are inhibited about 25% by radiation doses which previously had no apparent effect (90,000 r, 250 KV X-rays). This is partially reversed by addition of potassium ions and can be considerably enhanced by post-irradiation treatment of the yeast with a cation exchange resin (Dowex-50). In this case respiration is inhibited as well as fermentation. Starvation (24 hours aeration in distilled water) likewise increases the apparent effect of irradiation on both respiration and fermentation when these are measured in potassium- and phosphate-free buffer. Inhibitions of 50% are common. These can be increased still further by treating the starved yeast with ion exchange resin, and as much as 90% inhibition of fermentation is observed in such yeast after 90,000 r. Pre-irradiation treatment with the resin has much less effect. These results indicate a possible method for revealing radiation damage to metabolic processes ordinarily considered insensitive or not measured in the earlier experiments.

Abstract of Paper Presented at
the 38th Annual Federation Meeting
Atlantic City, New Jersey, April 11-14, 1954

EFFECTS OF PARATHYROID EXTRACT ON THE BONE LESION INDUCED BY BERYLLIUM COMPOUNDS

by
William L. Downs, Elliott A. Maynard and Harold C. Hodge

Weanling albino rats fed a diet containing 2.4% beryllium sulphate tetrahydrate for a period of one month develop a zone of hyperossification in the region of most active bone growth. This same lesion can be observed in weanling rats following the intraperitoneal administration of an aqueous suspension of beryllium oxide (2 gm./kg. body weight). The daily intraperitoneal administration of 40 USP units of parathyroid extract (PE) (total dose - 1020 units) failed to prevent or modify the simultaneous development of the beryllium-induced bone lesion following a single intraperitoneal injection. In this experiment the beryllium content of the bone was found to be significantly lower in the group of rats treated with PE (12 µgm. Be/gm. ash) than in the control group that received only beryllium oxide (25.2 µgm. Be/gm. ash). The daily intraperitoneal administration of 100 units of PE (total dose - 5200 units) did not cause resorption of the spongy bone laid down during the period of ingestion of a diet containing 2.4% beryllium sulfate. Massive single doses (1000 USP units) of PE failed to induce resorption of the spongy bone which was laid down following the intraperitoneal administration of an aqueous suspension of beryllium oxide 12 to 238 days previously.

Abstract of Paper Presented at
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THE ROLE OF BARTONELLA MURIS INFECTION IN MODIFYING THE RESPONSE OF RATS TO IRRADIATION FROM POLONIUM OR X-RAYS

by
J. N. Stannard and James K. Scott

The rickettsial infection, Bartonella muris, ordinarily latent, can be activated by splenectomy of animals harboring the disease. When activated it produces anemia and intravascular hemolysis with hematuria which might be expected to exacerbate the acute radiation syndrome when present. First it was found that intravenous injection of about 40 μc/kg of the radioactive element, polonium 210, a strong alpha emitter which concentrates in the spleen, can activate the infection in rats (using appearance of organisms in circulating red cells as a criterion) and thus imitate splenectomy. Then strains of rats harboring the infection endemically were compared to strains known to be free of it and also groups of carrier rats receiving chemotherapy (Mapharsen) prior to polonium injection were compared with controls receiving only polonium. The anemia was always more profound and hematuria much more frequent in the infected animals. Nevertheless, the mortality after polonium was no greater in infected groups, and mean survival time was even slightly longer. Thus the presence of active Bartonellosis does not necessarily increase mortality despite exacerbation of the anemia. Later it was found the x-irradiation (600 r, 250 KV) could also activate the infection but not as quickly nor as uniformly as polonium.

Abstract of Paper Presented at
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Atlantic City, New Jersey, April 11-14, 1954

IONIC EXCHANGE AND RECRYSTALLIZATION OF BONE MINERAL IN VITRO

by
John H. Weikel, Jr.

With the use of radioactive tracers of calcium and phosphorus, the mineral crystals of bone have been shown to undergo ionic exchange with ions of the surrounding aqueous medium. Subsequently, it was found that surface exchange could not account for all of exchange that takes place. The term recrystallization was introduced to describe the equilibration occurring between the bulk of the mineral crystals and solution. Recrystallization in vivo was first suggested by Hevesy as resulting from daily fluctuations in serum calcium and phosphate levels. However, it has been since demonstrated that spontaneous recrystallization occurs in vitro in solutions of constant composition. A kinetic study of a synthetic system (hydroxyapatite: inorganic buffers) was undertaken to elucidate the mechanism of the process. The most reasonable interpretation of the data obtained is that recrystallization does not involve a solution and redeposition of ions, but rather an interchange of ions by thermal vibration. It is necessary to assume "faults" in the crystal lattice for this to occur. Further, the rates of ionic interchange decrease markedly as isotopic ions penetrate deeper layers of the crystal. One must conclude that only a few "molecular" layers of the crystals can take part in equilibria over an observational period of a few weeks.

Abstract of Paper Presented at
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ON THE NATURE OF SODIUM IN BONE

by
William R. Stoll

The skeleton serves as a repository for a large proportion of the total body sodium. The nature of this skeletally bound sodium has never been adequately characterized. Sodium ion probably cannot substitute isomorphically for calcium within the crystalline lattice of the bone mineral. Rather, it appears likely that sodium resides in the crystalline surfaces and in faults and occlusions. Convincing quantitative evidence in support of this thesis has been lacking. An investigation of a synthetic system in vitro was undertaken to elucidate the mechanisms underlying the fixation of sodium by bone mineral. Hydroxyl apatite was equilibrated with inorganic buffers under a variety of conditions. Analyses for alkali metals were performed by flame photometry. It was found that sodium displaces calcium ion on an equimolar bases from the hydration layer of the mineral crystals. It appears likely that sodium ions may also displace some calcium ions residing in the crystal surfaces. While the magnitude of this surface exchange process is sufficiently large to account for the sodium found in bone, further information will be required to establish whether the exchange process is the only mechanism involved in the skeletal fixation of sodium.

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the 38th Annual Federation Meeting
Atlantic City, New Jersey, April 11-14, 1954

EFFECT OF MERCURIC CHLORIDE ON YEAST AND ON YEAST HEXOKINASE

by
L. Hurwitz and Emily Chaffee

The interaction of mercuric chloride with living yeast cells and with a purified preparation of yeast hexokinase was studied and compared. Very low concentrations of mercuric chloride were necessary to inhibit both the reaction of the enzyme preparation and the fermentation of glucose by yeast cells. The addition of mercury complexing agents, such as pyruvate and ATP, prior to the addition of mercury afforded protection against the inhibitory effects. However, in each case if the mercury were added before the complexing agent, much less protection was observed. For example, when 0.15 M pyruvate was added to yeast prior to mercury, no inhibition was observed; but when it was added 60 min. after mercury, the inhibition was 80%. Similarly, when ATP was added to hexokinase before the mercury, the inhibition was only 18%; but when ATP was added after the mercury, the inhibition was 90%. The observed effects cannot be due to an irreversible inactivation by mercury of the purified enzyme or of the active metabolic groups in the living yeast. Each reaction can be shown to be reversible by other agents. Cysteine can reverse the inhibition of hexokinase. Dialysis against a higher concentration of yeast can reverse the inhibition of glucose fermentation in yeast cells. Apparently carboxyl and phosphate containing complexing agents can prevent mercury inhibition, but cannot reverse it. On the other hand, sulfhydril agents can both prevent and reverse the effect. The mechanism of combination of mercury with the enzyme with the yeast cell is complex, and not well understood.

Abstract of Paper Presented at the 38th Annual Meeting
of the Federation of American Societies for Experimental Biology,
Atlantic City, New Jersey, April 12-16, 1954

EFFECT OF PHOSPHORUS-32 ON GROWTH AND VIABILITY OF RAT EMBRYOS

by
Melvin R. Sikov and Thomas R. Noonan

Effects of a beta-emitting radioisotope (P^{32}) on the course of pregnancy in the rat were studied by intraperitoneal injection of solutions of the isotope into pregnant female rats of 6, 8, 9 and 10 days gestation. Doses of 0.5, 1.0, 1.5 and 2.0 mc/animal were used. All animals were sacrificed on the 14th day of gestation. The number of living fetuses was compared with the number of implantation sites to ascertain the toxicity of the isotope. Length and weight measurements were made to determine if any retardation of development was produced by the isotope and the fetuses were examined for gross malformations. Certain fetuses were randomly selected and processed for radioactivity determinations to allow estimation of the radiation dose in roentgen equivalent physical received by each fetus. When 1.5 or 2.0 mc was injected on the 10th day of gestation, the percentage of embryos surviving until the 14th day was greatly reduced; neither 0.5 nor 1.0 mc produced any significant increase in mortality. Weight and length reduction was found at all doses, the reduction being greater with increasing dose. When injection was made on the 6th, 8th or 9th day, doses above 0.5 mc killed almost all of the fetuses. Animals injected with 0.5 mc on these days showed a great reduction in the percentage of the litter surviving to the 14th day. The size of these fetuses was found to be reduced in all cases.

Abstract of Paper Presented at
the 38th Annual Federation Meeting
Atlantic City, New Jersey, April 11-14, 1954

MECHANISMS OF UPTAKE OF PHOSPHATE BY YEAST CELLS

by
Joan Wright Goodman and Aser Rothstein

Orthophosphate is taken up by living yeast cells if glucose or mannose or fructose is present. However, other metabolizable substrates such as alcohol, lactate or pyruvate do not invoke any such response. Studies of the effect of extracellular pH indicate that only the monovalent ion, $H_2PO_4^-$, is taken up. The uptake of orthophosphate in the presence of glucose has been measured both chemically and by isotope technique. The relative specific activity of the extracellular inorganic phosphate remains constant during the course of the uptake; there is no measureable exchange. In other words, there is an inflow but no outflow of phosphate. During the uptake the inorganic orthophosphate concentration of the cell does not change significantly, and the inward flow of phosphate occurs against a concentration gradient as high as 100-1. Although phosphate uptake requires the presence of glucose, there is no obvious stoichiometric relationship. In fact, glucose uptake proceeds rapidly in the absence of phosphate. On the other hand, the rate of phosphate uptake increases asymptotically with increasing extracellular phosphate concentration. An active transport of phosphate, involving a combination of phosphate with constituents of the cell membrane, must occur. The energy for the transport is derived from glycolytic reactions. The absence of exchange indicates that the membrane is impermeable to inorganic orthophosphate and that the transport mechanism is effectively irreversible.

Abstract of Paper Presented at
the Seventy-Second Annual Federation Meeting
Atlantic City, New Jersey, April 12-16, 1954

LYSINE METABOLISM AS STUDIED BY IN VIVO METABOLITE-OVERLOADING

by
Morton Rothstein and Leon L. Miller

Data obtained by utilizing the in vivo "metabolite-overloading" technique reveals that one of the first steps in lysine metabolism in the rat involves the formation of pipecolic acid. The procedure involves the simultaneous intraperitoneal injection into a rat of a large amount (about 0.4 gm) of a suspected catabolite and an isotopic precursor. The former compound mixes with any similar biologically formed material and may be isolated from the urine in large amount. By this method, highly radioactive pipecolic acid has been isolated after administration of lysine-ϵ-C^{14}. Similarly lysine-ϵ-N^{15} yields pipecolic acid containing excess N^{15}, indicating that the α-amino group of lysine is removed first. It is probable that pipecolic acid is an intermediate between lysine and α-aminoadipic acid, although no reverse formation of pipecolic acid from α-aminoadipic acid-ϵ-C_{14} takes place. Further studies have clarified the pathway by which lysine, via glutarate forms glutamic acid. Evidence has been obtained for the sequence: glutarate $\longrightarrow$ glutaconate $\longrightarrow$ L-α hydroxyglutarate $\longrightarrow$ α-ketoglutarate $\longrightarrow$ glutamate. The pathway is partially reversible under the conditions of our experiments, as acetate-1-C^{14}, probably via

α-ketoglutarate, yields radioactive L-α-hydroxyglutarate, but not glutaconate or glutarate. Evidence in favor of δ-aminovalerate as a metabolite of lysine is provided by the fact that δ-aminovalerate -δ-C^{14} yields extremely radioactive glutaric acid. However, this amino acid does not lead to radioactive formate and is therefore not the source of the small amount of the latter compound derived from lysine-ε-C^{14}.

Abstract of Paper Presented at
the Seventy-Second Annual Federation Meeting
Atlantic City, New Jersey, April 12-16, 1954

BIOSYNTHESIS OF HEMIN IN SOY BEAN ROOT NODULES

by
Jonas E. Richmond and Kurt Salomon

It has been shown by others that the root nodules of leguminous plants contain a hemo-protein with a prosthetic group indistinguishable by the methods used from protohemin IX of hemoglobin. The present investigation is concerned with studies on the biosynthesis of this pigment in soy bean root nodules. The capacity of soy bean nodules and homogenates to incorporate the carbon atoms of glycine and acetate into hemin was determined using various media for homogenization and incubation. Nodule homogenates, similar to bone marrow homogenates, were capable of incorporating the carbon atoms of acetate and the alpha carbon atom of glycine into the tetrapyrrole structure. In order to study the relation of hemin synthesis to the tricarboxylic acid cycle in plants, experiments were carried out using substances such as pyruvate, oxaloacetate, alpha-keto glutarate and others. The incorporation of the carbon atoms of acetate and the alpha carbon atom of glycine into hemin as well as the production of carbon dioxide were found to be functions of the substrates present. The effect on hemin synthesis of specific inhibitors of respiration, glycolysis and the citric acid cycle were also studied. The results obtained indicate that the root nodule system and bone marrow homogenates differed in their respective responses to some of the inhibitors used.

Abstract of Paper Presented at
the Seventy-Second Annual Federation Meeting
Atlantic City, New Jersey, April 12-16, 1954

PHOSPHATE METABOLISM IN NORMAL AND PATHOLOGICAL MAMMALIAN ERYTHROCYTES

by
T. A. J. Prankerd and K. I. Altman

The uptake and distribution of phosphorus in esters of mammalian erythrocytes has been studied during incubation of fresh blood with $NaH_2P^{32}O_4$ at 37°C. Constant pH mixing and oxygenation were obtained by introduction of 95% O_2 and 5% CO_2. Tricloracetic acid extracts from standard volumes of packed cells were made at varying times and analysed by 2 dimensional paper chromatography according to Caldwell (Biochem. J. 55: 4, 58). The organic and inorganic phosphorus compounds were located with a scanning counter, the relative Rf values being known and radioactivity and total phosphorus estimated. The uptake and distribution of P^{22} within phosphate fractions of normal human erythrocytes remains reasonably constant, equilibrium being reached after 4 to 6 hr. Plots of relative specific activities (R.S.A.) against time show that the most active fraction is 2,3-diphosphoglyceric acid which bears a precursor relationship to ATP. After 6 hr. incubation phosphorus tends to escape from normal cells and the activity of the esters falls. This change is hastened by fluoride and iodoacetate, the former producing a marked fall in the RSA of ATP, the latter a fall in RSA of all fractions with loss of cell phosphorus. Deterioration of normal cells during incubation is prevented by adenosine. Certain pathological cells show this effect to a

greater extent, and in these guanosine acts similarly but adenine does not. It is concluded that the carbohydrate moiety of these nucleosides is the active agent and is effective, after phosphorylative cleavage, in replenishing some of the essential carbohydrate stores of the erythrocyte to participating via the hexose-6-phosphate shunt.

Abstract of Paper Presented at
the Radiation Research Society Meeting
Cleveland, Ohio May 17-19, 1954

LIFE SPAN REDUCTION IN RATS CAUSED BY HIGH SINGLE VS. DIVIDED DOSES OF X-RAY

by
John B. Hursh and George Casarett

As an extension of an earlier experiment (T. Noonon, F. Van Slyke, J. Hursh. University of Rochester Report, UR-161 (1951)) which appeared to show that for single doses of 600, 300, and 150 r mean survival times of rats were reduced by an amount proportional to the dose, an experiment was devised in which Wistar strain male rats received either zero dose, 600 r as a single dose, 60 r per day for 10 consecutive days, or 20 r per day for 30 consecutive days. Each group contained 50 rats, 31 to 33 weeks old at time of irradiation. The animals were irradiated at 18.4 r per minute. The period of observation extended until death of all the rats. Excluding the few rats that died within 60 days after irradiation, the average life span of the unirradiated rats was found to be 630 days, of the 600 r single dose was 510 days, of the 60 r per day for 10 days was 593 days, and of the 20 r per day for 30 days was 644 days. It is concluded that the shortening of life span is significantly greater when 600 r is delivered as a single dose than when a total of 600 r is delivered according to the above divided dose schedules.

Abstract of Paper Presented at
the Second Annual Meeting of the Radiation Research Society
Cleveland, Ohio, May 17-19, 1954

THE BINDING OF POLONIUM BY RED CELLS AND PLASMA

by
Robert G. Thomas and J. N. Stannard

The alpha emitter polonium is maintained at a relatively high concentration in the blood over long periods following administration. To learn the manner in which this radioactive element is carried in the circulation, analyses were carried out on red cells and plasma. In line with earlier work, a much greater concentration was found in the cellular fraction, and the ratio of cell to plasma activities can reach quite high values ($\geq$20). In vitro studies indicate a similar partitioning. Upon hemolysis, almost all the red cell polonium appears in the hemolysate with very little in the stroma. Furthermore, it appears almost quantitatively in the hemoglobin crystallized from such an hemolysate and several washings and recrystallizations do not reduce the alpha activity per gram dry weight. Preliminary zone electrophoretic experiments show migration of the polonium with hemoglobin. Splitting of the crystallized hemoglobin shows no activity to be associated with the porphyrin ring but an apparent binding of the metal to the globin. Analyses of the total lipids isolated from both cells and plasma show no measurable radioactivity. At the low concentrations seen in plasma almost all of the polonium was associated with the fractior precipitated by trichloroacetic acid. Zone electrophoretic studies show polonium in each fraction and indicate a fairly uniform distribution among the different plasma proteins.

Abstract of Paper Presented at
the American Industrial Hygiene Association Meeting
Chicago, Illinois, April 24-28, 1954

THE ACUTE TOXICITY OF INHALED RADON

by

Donald A. Morken

Mice of CAF_1 strain were exposed to high concentrations of radon in a specially constructed inhalation system. The system was designed to provide a high degree of control over the radon atmosphere. The concentration of radon was about 2.2×10^{-4} curie per liter and the atmosphere was essentially free of the daughter products of radon. The 30-day LD-50 is represented by an exposure for one hour to a concentration between 5.7 and 8.8 millicuries per liter.

Autopsy revealed a generalized radiation damage throughout the body. Changes in cellular composition of the blood were not as pronounced as in similar exposures to x-irradiation. The physiological effects reported in the literature were observed in these mice.

Abstract of Paper Presented at
the American Industrial Hygiene Association Meeting
Chicago, Illinois, April 24-28, 1954

INVESTIGATIONS INTO THE TOXICITY OF POLONIUM: EFFECTS OF ROUTES OF ADMINISTRATION

by
F. A. Smith, Paul E. Morrow, L. J. Casarett,
R. J. Della Rosa, and J. N. Stannard

Different patterns of absorption, distribution and excretion were obtained in thirteen cats, following administration of two forms of polonium by various routes. These patterns were determined chiefly by the route of administration. Following administration into the duodenal loop, the greater portion of the absorbed dose appears in the liver, kidneys, lung and spleen, whereas after pouch administration the bulk of the absorbed material appears in other tissues. Intravenous administration results in a distribution pattern intermediate between those of the other routes. Considerable absorption from the stomach was achieved in all cases, and under certain circumstances (with neutralized polonium) exceeded that from the loop.

Abstract of Paper Presented at
the Conference on Permissible Bone Burdens,
Salt Lake City, Utah, June 18-19, 1954

NATURAL RADIUM CONTENT OF THE HUMAN BODY

by
John B. Hursh

A review was given of the available data on the natural radium content of the human body. The following conclusions were arrived at. The first is that, earlier data to the contrary, there is no good reason to believe that naturally acquired radium has been demonstrated to exceed 10^{-10} grams radium 226 per whole body. The second is that Ra^{226} is the major naturally acquired alpha emitter in the human body, and that analysis of this radium isotope serves to adequately account for the alpha emitter background radiation. A third conclusion is that present equipment measuring gamma-radiation from the living human subject as a source is insufficiently sensitive to measure naturally occurring levels of Ra^{226}.

This report designated UR-343 is to be published in the Archives of Biochemistry and Biophysics but will not receive standard A.E.C. distribution.

THE METABOLISM OF δ-AMINOVALERIC ACID-δ-C^{14}

by

Morton Rothstein and Leon L. Miller

The intermediary metabolism of δ-aminovaleric acid-δ-C^{14} has been studied in the intact rat. This amino acid is apparently rapidly oxidized with about 65% of the C^{14} of an intraperitoneal dose appearing in the expired carbon dioxide within 6 hours.

The in vivo metabolite overloading technique was used with the simultaneous injection of a small dose of δ-aminovaleric acid-δ-C^{14} and large doses of glutarate, formate, and ornithine with the isolation of the latter from the urine. The isolated glutarate was highly radioactive, a finding in keeping with the view that δ-aminovaleric acid is first deaminated probably to glutaric semialdehyde which is then converted to glutaric acid.

This report, designated UR-340, is to be published in the American Journal of Anatomy but will not receive standard A.E.C. distribution.

THE DEVELOPMENT OF THE HAMSTER LOWER INCISOR AS OBSERVED BY ELECTRON MICROSCOPY

by
Michael L. Watson and J. K. Avery

Abstract

Thin sections of undecalcified developing tooth were studied.

In the dentine it was found that: (1) The predentinal odontoblastic process is a true extension of the odontoblast, enclosed by the cell wall and filling the predentinal tubule. (2) The predentinal-border apparatus lies at the surface of the predentine and within the odontoblasts. (3) Mitochondria and endoplasmic reticulum are found only on the pulp side of the predentinal border apparatus, although the odontoblast cytoplasm extends into the predentine without a break. (4) Collagen fibers of predentine are smallest (ca. 100Å) near the predentinal border and tubular walls where they are apparently formed and increase in diameter (ca. 600Å) towards the calcification front.

In enamel: (1) The findings of John Tomes (1849) for rod orientation in the rodent enamel are confirmed. (2) Enamel appears to be intracellular. (3) The wall of the inner enamel rod is deposited first and is followed by the core. (4) Calcification is gradual, and most enamel is at least lightly calcified. (5) Crystals are ribbon-shaped with dimensions in older enamel of 100Å by 500Å by

5 μ to 10 μ. (6) The crystals have a complex and high degree of preferred orientation determined by the similar organization of the matrix. (7) The c-axis of the crystal lattice is parallel to the long crystal axis.

TECHNICAL REPORTS ISSUED FOR STANDARD DISTRIBUTION

April 1, 1954 thru June 30, 1954

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UR-300	Tyndallometric Estimation of Polydisperse Aerosol Deposition in Man (UNCLASSIFIED) Issued: April 4, 1954	Hilcken	Health and Biology
UR-321	The Effects of X-radiation on the Metabolism of Bakers' Yeast (UNCLASSIFIED) Issued: June 30, 1954	Bair	Health and Biology
UR-323	The Synthesis of δ-Aminovaleric Acid-δ-C^{14} (UNCLASSIFIED) Issued: April 27, 1954	Rothstein	Chemistry General
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UR-329	The Effects of a Maintained Body Burden of Polonium in Rats. I. Pilot Distribution and Excretion Experiment (UNCLASSIFIED) Issued: May 18, 1954	Stannard	Health and Biology
UR-330	Quarterly Technical Report January 1 thru March 31, 1954 (UNCLASSIFIED) Issued: June 22, 1954	Blair	Health and Biology