THE COURSE OF VITAMIN B_1 METABOLISM IN MAN AS INDICATED BY THE USE OF RADIOACTIVE SULFUR, A NEW SYNTHESIS OF 4-METHYL-5-β-HYDROXYETHYL-THIAZOLE, AND A DEMONSTRATION OF THE USE OF ANTI-COINCIDENCE METHODS IN RADIOACTIVITY DETERMINATIONS

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ABSTRACT

Thiamin synthesized from radiosulfur S^{35} was injected intramuscularly in two series of experiments, using a human subject on a normal and a B_1-free diet. Determinations of the free B_1 (by the thiochrome method) in the urine, the radiosulfur in the inorganic, ethereal, and neutral fractions of the urine, and the total radiosulfur in the feces were made. Rapid destruction of injected thiamin was indicated in both experiments by the appearance of radiosulfur in the inorganic fraction of the urine. Rapid interaction of the injected material with that pre-existing in the tissues was indicated by the appearance of free thiamin in the urine, without corresponding active sulfur. Additional destruction of thiamin was indicated by an excess of the neutral radiosulfur activity over the free B_1 after the injections were stopped. After 36 days of the B_1-free diet the injection of 8 mg. over a period of 3 days resulted in the excretion of 0.8 mg. of pre-existing thiamin. On the normal diet the injection of 63 mg. over a period of 4 days resulted in the excretion of 16 mg. of pre-existing thiamin.

I. INTRODUCTION

The function of thiamin in the biochemistry of the various life processes is becoming increasingly clear, and its behavior in the carbohydrate metabolism is being studied in considerable detail. One of the important problems which is not necessarily connected with the function of the vitamin itself is the question of what happens to thiamin in the human body, as contrasted with what it does. By definition a vitamin is a substance which must be provided in the food, and usually cannot be synthesized in the body; the fact that it must be supplied indicates that there are processes which result in its removal as an effective part of the biochemical processes. This removal is most likely to be the result of either or both of two separate kinds of process: (1) the direct excretion of essentially unchanged thiamin or (2) the alteration or destruction of thiamin.

The evidence that the excretion process takes place to a considerable extent is quite clear, but only a fraction of the intake can be accounted for in this way. The literature on the thiamin excretion of normal human subjects might be summarized as follows: With five subjects Jollifee (1) found that from 7-28% (with an average of 15%) of the total ingested thiamin appears in the urine, the total intake ranging from 470-4300 micrograms. The average 24-hour urinary excretion of 500 micrograms for the control diets, which

were fairly high in thiamin content (3 mg.), are in keeping with the values reported by others for "normal" diets of unknown vitamin content, where urinary excretions ranging from 30-500 micrograms per day have been observed (2, 3, 4, 5, 6, 7). The fecal excretion on such normal diets seems to be about 180 micrograms (6), amounting to about another 10%; the total recovery is thus about 25% of the intake.

Most of the experimental work has been concerned with testing for "B_1 saturation" or identifying clinical deficiencies, and consequently is concerned with the excretion of extra added thiamin above the normal or average amounts. The urinary excretion of orally added thiamin ranges from 5-35%, the general average being about 18% (2, 3, 6); the fecal recovery is of the same order of magnitude (6). With parenterally administered extra thiamin the urinary excretion is about the same (7, 8).

In general, then, at least half of the thiamin intake is not excreted as such under any normal conditions. It seems unlikely that there exists any slow or rate-limiting process in the attainment of equilibrium with respect to the excretion of free thiamin in these experiments; certainly equilibrium would have been reached in the "normal diet" cases. In the work where large added amounts of thiamin were given, the experimental periods continued for as long as a month, and these facts, together with the fact that clinical symptoms of beri-beri can be produced fairly rapidly (1) seem to rule out completely the possibility of any large special storage of the vitamin other than that normally in the various tissues.

Only in the rat has a more complete investigation been made (9, 10). The total storage of thiamin in the various tissues was determined after the rats had been on diets containing various amounts of thiamin for long periods, and it was found that the total reserves on an "average" adequate diet amounted to about 35 International Units, which could be increased to 75 I.U. on high-vitamin-content diets. At least 80% of the extra amount appeared in the liver and muscle tissue. No increase in reserves beyond this limit was obtained with a two hundred fold increase in the intake. The excretion of thiamin during experimental periods of about 10 weeks, at intake levels ranging from 0-704 I.U. per day, and the total stores were also determined. An intake level of about 7 I.U. per day was required to maintain the initial normal reserve of 35 I.U. The relation between intake and excretion indicated that for an intake greater than 7 I.U. per day the urinary excretion is very nearly a linear function of the intake up to an apparent limit of 100 I.U. per day; the fecal excretion is a linear function of intake above this limit. Considering only the high-intake range, the total excretion was almost exactly 94% of the intake, which indicates that some 6% of the total intake is unaccounted for. In view of the lack of any increase of body reserves on such high-intake diets, the unaccounted-for 6% must have been altered or destroyed in some manner. That the destruction process exists at all levels of intake in the rat is indicated most clearly by the data on the reserves: The average daily loss of thiamin from the normal reserve of 35 I.U. was computed from the difference between the total available (total intake + initial total reserve) and the total amount accounted

for (total output + final total reserve at the end of the experimental period). A destruction of 1.5 I.U. per day was found for a thiamin-free diet, with an increase up to 50 I.U. per day on the diets containing very large quantities of thiamin.

The particular focus of the work described below is an examination in more detail of these general basic facts as applied to human metabolism; the problem is one ideally suited to the use of radioactive-tracer techniques, since it would be possible to determine the over-all behavior as well as any destruction or degradation processes. The main points of the research are covered by the published work, Part II of this thesis; Part III contains the details of the experimental work itself, and Part IV some other contributions which were an outgrowth of the study.

II. SUMMARY AND CONCLUSIONS

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THE COURSE OF THIAMIN METABOLISM IN MAN AS INDICATED BY THE USE OF RADIOACTIVE SULFUR[*,†]

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When supplementary thiamin (Vitamin B_1) is ingested or injected, the amount which appears in the urine during the succeeding twenty-four hours usually increases, but the total additional excretion always falls short of the supplement, even when the vitamin is injected and there can be no question of incomplete absorption. We have sought information on this unaccounted-for moiety by synthesizing thiamin from sulfur which contains the radioactive isotope S^{35} (designated as B_1^*) and by following, after injection of the B_1^*, the excretion of the radiosulfur (S^*) in the urine and feces and the excretion of total free B_1 in the urine.

Radiosulfur was prepared by bombarding elementary sulfur with 7.5 MEV. deuterons, and thiamin bromide hydrobromide containing S^{35} was synthesized from the bombarded sulfur.[1]

The half-life of radiosulfur is about 88 days and it emits negative electrons with a maximum energy of 0.107 MEV.[2] This long half-life makes extended observations possible; some samples showed measurable activity 18 months after the radiosulfur had been prepared.

The radioactive vitamin was injected intramuscularly in two series of experiments; in the first series the subject, a young man in good health, was on a vitamin B_1-free diet for 36 days prior to the first injections and for the 15 days of the experimental period; in the second series the same subject was on a normal diet. Essentially the same results were obtained in both series.

Free (unphosphorylated) vitamin B_1 was determined in the urine by a modification of the thiochrome method. The urinary sulfur compounds were fractionated into the inorganic sulfate, ethereal sulfate and neutral sulfur components, the sulfur of each component was converted into elementary sulfur,[3] and the radioactivity of the sulfur was then measured quantitatively by comparison with a standard sample of the same radiosulfur. In the feces only the total radiosulfur was determined.

The radioactivity measurements were made with open, coincidence Geiger counters. The sulfide-coated copper counter tubes and the samples were enclosed in a large partially evacuated bell jar, provided with an externally operated arrangement for moving the samples successively to a definite position in front of the counters. The standard Rossi coincidence

circuit was used in the amplifiers; these had time constants of 3×10^{-4} seconds. It was possible to measure samples as weak as 3% of the background by making very long runs.

Separate experiments showed that B_1* added to urine does not exchange its radiosulfur with the inorganic or ethereal sulfur compounds; the S* was quantitatively recovered from the neutral sulfur fraction.

Figure 1 shows the daily excretion of radiosulfur (all forms) in the urine and feces after a daily intramuscular injection of 16 mg. of B_1* for four

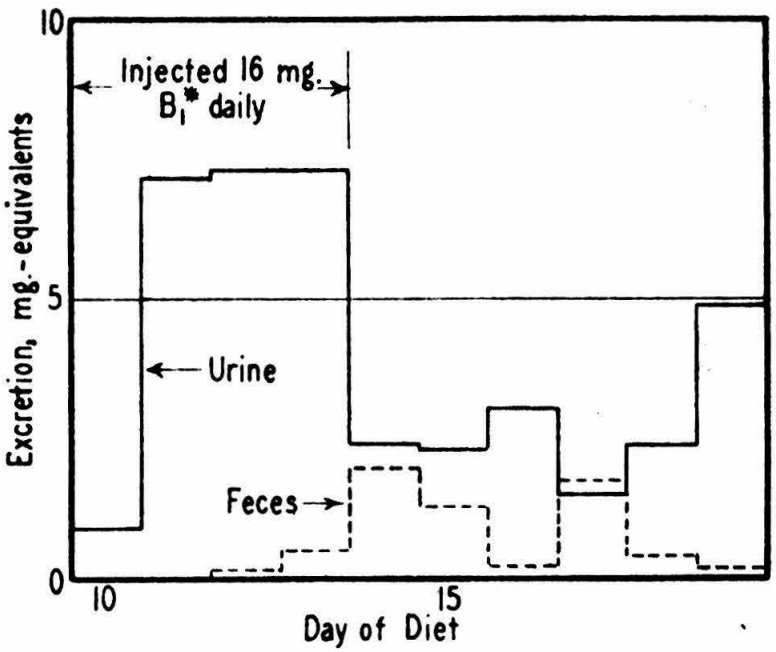

FIGURE 1

The daily recovery of radiosulfur (calculated as mg. of vitamin B_1) after intramuscular injection of B_1*; normal diet.

days, while the subject was on the normal diet. Six days after the last injection a total of 61% of the injected radiosulfur had been recovered from the urine and 11% from the feces; 28% of the injected material remained unaccounted for. It is clear from the low and diminishing excretion in the feces that the urine is the major excretory medium of parenterally administered B_1 and its decomposition products.

Figure 2 shows the daily excretion of free thiamin in mg. and of the radiosulfur present in the neutral sulfur fraction expressed in terms of mg. of B_1*. If only pure B_1* is present in urine these two should be the same;

consequently the excess of thiamin over radiosulfur shown in the figure indicates excretion of thiamin already present in the body. Since the total amount of B_1 in the blood in this case could hardly have exceeded 1 mg.[4] the excess of 7.5 mg. of non-radioactive vitamin in the urine on the first day of the injections must have come from the tissues. This indicates that the injected vitamin interacts very rapidly with that preëxisting in the tissues.

Although the whole of the neutral radiosulfur in the urine during the injection period shown in the figures may have been vitamin B_1, this is im-

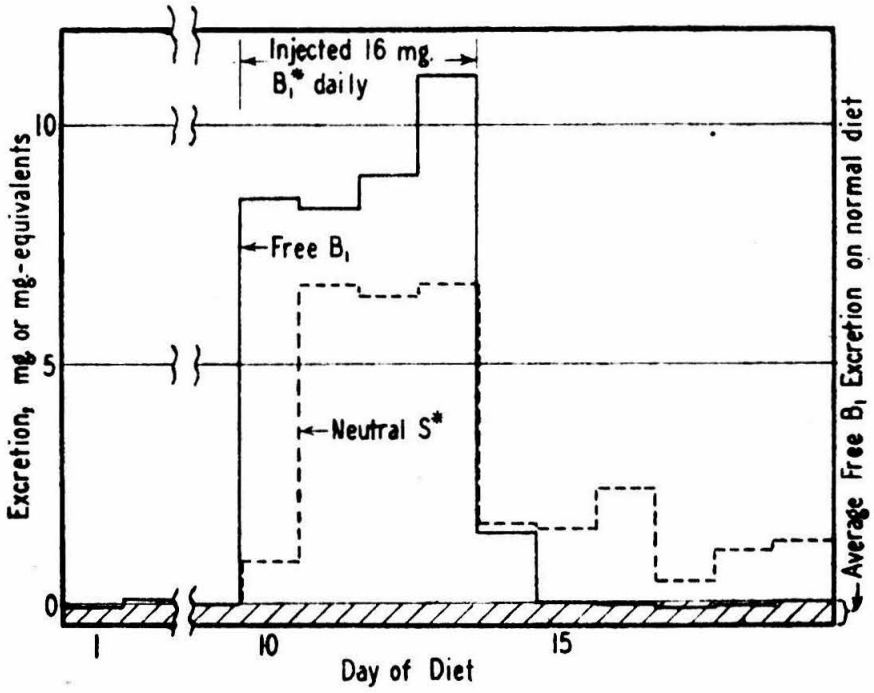

FIGURE 2

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of B_1^*; normal diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

probable in view of the findings on the later days. The difference between the total free B_1 and the total neutral radiosulfur is certainly the minimum amount of preëxisting vitamin which was displaced from the tissues.

During the six days following the termination of the radiothiamin injections the neutral radiosulfur in the urine exceeded the thiamin excretion. The difference between the neutral radiosulfur and the free B_1 sulfur is the minimum amount of neutral sulfur-containing decomposition products of the thiamin which was excreted. The continued excretion of radio-

sulfur is evidence that tissue thiamin is continuously undergoing destruction at a rapid rate.

These features were shown even more clearly in the series of experiments when the subject was on the B_1-free diet and 2.7 mg. of B_1* were injected daily from the 37th to 46th days, inclusive, of the B_1-free diet (Fig. 3). Thiamin excretion in the urine increased immediately, but no radiosulfur was detected in the urine for the first two days (less than 20 γ). The entire increment in the urine came from preëxisting thiamin in the tissues which was displaced by the injected material, again indicating that injected B_1

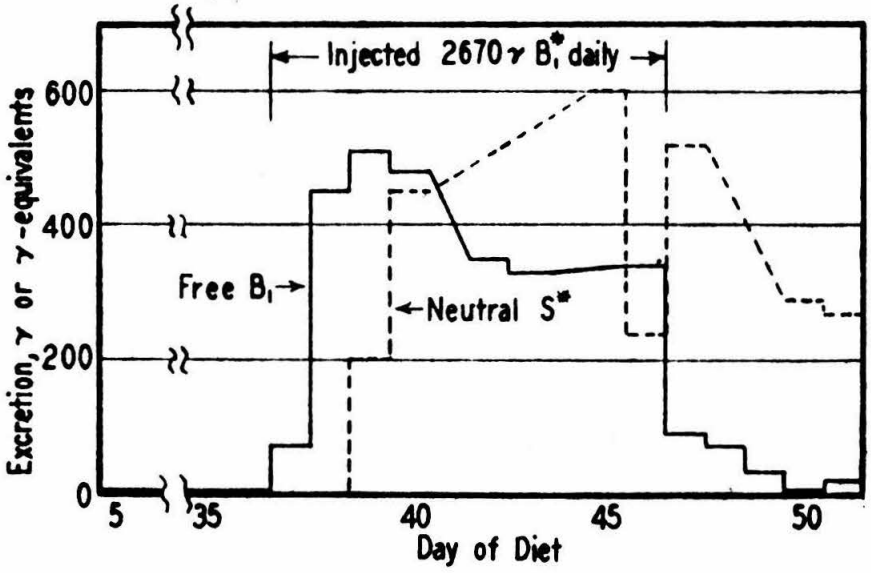

FIGURE 3

The daily excretion of neutral radiosulfur and free thiamin in the urine after intramuscular injection of B_1*; B_1-free diet. The radiosulfur has been calculated in terms of mg. of vitamin B_1.

rapidly enters the tissues from the blood. Since no thiamin was detectable in the urine for 30 days prior to the injections, the total quantity in the blood must have been less than 0.2 mg. For a short time at least after the injection of 2.7 mg., the ratio of radiothiamin to normal thiamin in the blood was probably greater than 1. If the excreted vitamin is an "overflow" moiety excreted before it enters the tissues, it should have been accompanied by a corresponding radiosulfur activity, but this was not found to be the case. The injected thiamin, therefore, must have entered the tissues very rapidly and interacted with the considerable quantities of thiamin already present.

Table 1 is a summary of some of the data illustrating this feature of thiamin metabolism. It is interesting that any free thiamin was displaced from the tissues of a subject who had been for 36 days on a B_1-free diet.

TABLE 1

THE DISPLACEMENT OF PREËXISTING THIAMIN FROM THE TISSUES BY ADDITIONAL THIAMIN INJECTED INTRAMUSCULARLY

DIET	INJECTION AND EXCRETION PERIOD (DAYS)	THIAMIN INJECTED (MG.)	MINIMUM AMOUNT OF PREËXISTING THIAMIN EXCRETED (MG.)
B_1-Free	3	8	0.8
Normal	1	16	7.5
Normal	4	64	16.0

The neutral sulfur and thiamin data in the two experiments are summarized in figure 4 which shows the difference between the thiamin and the neutral radiosulfur excretion. During the injection period the deficit of neutral radiosulfur indicates the minimum amount of preëxisting vitamin displaced from the tissues; the excess of neutral radiosulfur found in the urine after stopping the injections represents destroyed vitamin.

Figure 5 summarizes the urinary excretion of radiosulfur as inorganic sulfate; the radioactive sulfur appeared in this form in small quantities on the second day of the injections, and increased to considerable amounts by the end of the experiment, which is proof of the destruction of the injected vitamin and oxidation of the thiazole ring. The SO_4* from this oxidation mixes with the large amount of SO_4 in the body;[5] the excretion of the radiosulfur is therefore delayed. The oxidation of the thiazole ring was therefore probably much more extensive than is indicated by the amount of SO_4* in the urine in the first few days after beginning the injections.

The amounts of radiosulfur found as ethereal sulfate were in most cases very close to the experimental error of the radioactivity measurements; only a very small fraction of the radiosulfur appears in this form, and it may have arisen from exchange with the inorganic sulfate.

The extensive destruction of B_1 in the body is indicated in table 2.

TABLE 2

DESTRUCTION OF INTRAMUSCULARLY INJECTED THIAMIN

	TOTAL RECOVERY OF S* FROM B_1* (% OF TOTAL INJECTED)		PER CENT OF RECOVERED S* IN THE URINE WHICH REPRESENTS DESTROYED VITAMIN		
	FECES	URINE	AS NEUTRAL S COMPOUNDS	AS INORGANIC SULFATE	TOTAL
B_1-Free	..	26	21	21	42
Normal	11	61	18	25	43

Summary.—1. There is a rapid interaction of injected B_1 with that present in the blood and tissues.

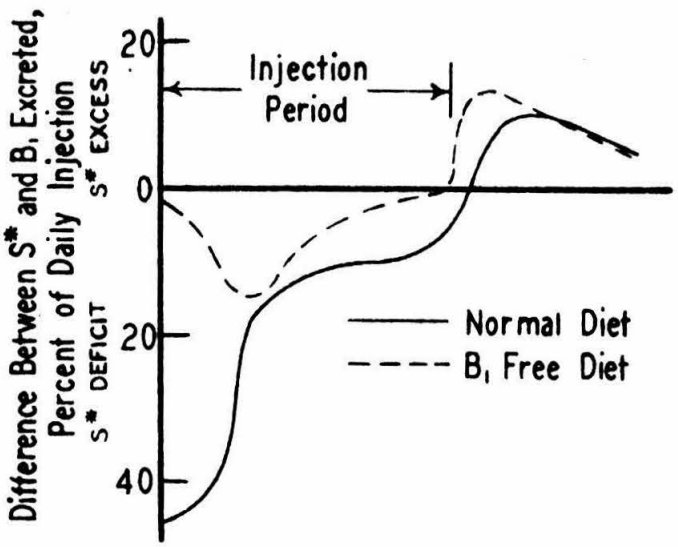

FIGURE 4

The difference between neutral radiosulfur and thiamin excretion after the injection of B_1*.

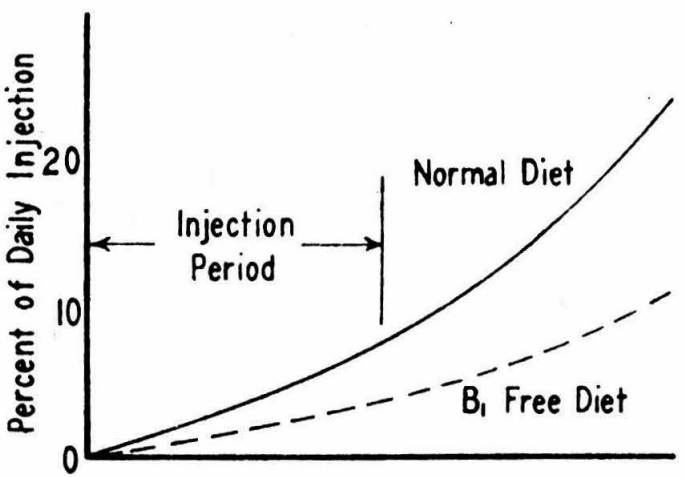

FIGURE 5

The urinary excretion of radiosulfur as inorganic sulfate after the injection of B_1*.

2. The displacement of preëxisting thiamin by injected thiamin demonstrates that a significant amount of the vitamin remains in the tissues even after 36 days of a B_1-free diet; larger quantities are present under normal nutritional conditions. This does not imply that the amount retained after a prolonged B_1-free diet is an adequate protective amount of this vitamin.

3. The metabolism (interchange and destruction) of vitamin B_1 is rapid and thus resembles that of the main metabolites—protein, fat and carbohydrate.

4. The rapid destruction of thiamin yields in the urine neutral sulfur compounds and inorganic sulfate.

5. The losses incurred by excretion and destruction are inevitable in the maintenance of a physiologically adequate concentration of thiamin and cocarboxylase in the blood and tissues.

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† This paper was presented at the meeting of the American Society of Biological Chemists in New Orleans, March 14–16, 1940.

[1] Cf. E. R. Buchman, *Jour. Am. Chem. Soc.*, **58**, 1803 (1936); J. K. Cline, R. R. Williams and J. Finkelstein, *Ibid.*, **59**, 1052 (1937).

[2] W. F. Libby and D. D. Lee, *Phys. Rev.*, **55**, 245 (1939).

[3] R. A. Cooley, Don M. Yost and Edwin McMillan, *Jour. Am. Chem. Soc.*, **61**, 2970 (1939).

[4] Robert Goodhart and H. M. Sinclair, *Jour. Biol. Chem.*, **132**, 11 (1940).

[5] Henry Borsook, Geoffrey Keighley, Don M. Yost and Edwin McMillan, *Science*, **86**, 525 (1937).

III. EXPERIMENTAL DETAILS

A. Thiochrome Assay in Urine

The determination of free thiamin in the urine samples was made by a procedure based on the general method developed by Jansen (11), Westenbrink (12), and Hennessay and Cerecedo (13). This method was first used at the California Institute by Eric Heegard and was modified for this work to make it adaptable to small samples, so that most of the urine could be used for the radioactive determinations. The use of pure thiochrome standards was made possible by the kindness of Dr. Edward Matzgar, who provided a portion of a sample of pure crystalline thiochrome obtained from Merck and Company. The final process used was as follows:

0.1-2.0 ml. of urine (containing about 1 microgram of B_1) was diluted to 200 ml. in a small flask, and the pH was brought to 4.1 by adding 5 ml. of a concentrated acetate buffer. About 1 g. of commercial Decalso, a synthetic zeolite (supplied by the local agents of the Permutit Company) which had been ground to 50-100 mesh and ignited, was next added; the flask was shaken and allowed to stand for 30 minutes with occasional shaking. The zeolite was then filtered out, using a small Buchner funnel and very little suction at first, with precautions to shake the flask and pour the mixture slowly so that the zeolite built up its own filter bed on the paper. It was next washed and dried with small amounts of ethyl

alcohol and ethyl ether and could then be quantitatively removed from the filter paper with the aid of a small brush.

The zeolite was weighed and divided into aliquots (usually four or more), which were placed in small (16x125mm.) thick-walled Pyrex test tubes. 2 ml. of 25% KCl was then added to each tube, and the tube was briefly shaken. Next 1 ml. of CH_3OH was added to each tube; then to one tube 1 ml. of water (for a blank) and to the remaining tubes varying quantities of 0.1% $K_3Fe(CN)_6$ solution and water to bring the total volume added to 1 ml. in each case. For 1 microgram of thiamin the quantity of ferricyanide was about 0.08 ml., the usual procedure being to add 0.06, 0.08, and 0.10 ml. if working in this range.

The quantity of ferricyanide required for particular amounts of thiamin was found to be critical, and an apparent change was noted in the ferricyanide solution on standing for 2 or 3 days. For these reasons that it was not possible to standardize on one quantity of ferricyanide, and it was necessary to provide sufficient aliquots to make certain that the maximum fluorescence was obtained from the particular absorption. It was not necessary to restrict the sample to 1 microgram of thiamin; this was the minimum for which any degree of accuracy was obtained. The relation between ferricyanide and thiamin was not, apparently, linear. (This may be a function of other reducing constituents in the sample.) Up to the amounts required for maximum fluorescence, however, small changes in the quantity produced sizable variations, and a tenfold excess decreased the thiochrome by only about 10-20%.

After the addition of ferricyanide and water 1 ml. of 30% NaOH and 5 ml. of isobutyl alcohol were added to each tube, and the tubes were shaken vigorously to mix the contents thoroughly. For this purpose it was found convenient to grind the tops of the test tubes flat and hold a small flat ground plate over the top for the shaking process. With care a completely leakproof seal was made, and the loss of solution was kept to a minimum by sliding the plate off again with a wiping motion; ground stoppers of the usual type resulted in larger losses of solution, and rubber stoppers must of course be avoided, since they contain large quantities of fluorescent extractable materials. The tubes were then centrifuged briefly to separate the two layers, and the isobutyl alcohol was drawn off with a small dropper or pipet. Another 5 ml. portion of isobutyl alcohol was added, shaken, centrifuged, and drawn off. It was found that 95% of the thiochrome from the usual urine samples was extractable in this way, and most determinations used this percentage as a factor; in special cases more extractions, to recover 100%, were made, but there seemed to be no particular advantage. Provided the exact volumes were maintained so that the total volume of the extracted alcohol did not vary, it was unnecessary to determine the exact volume of extract in each case when dilution was necessary to bring the fluorescence within range of the standards.

The isobutyl extract of thiochrome was placed in thin-walled Pyrex test tubes, and the concentration was determined by simple visual comparison of the fluorescence with that of standards made up from known quantities of pure thiochrome in isobutyl alcohol in

identical sealed tubes. The set of standards contained 20 tubes, with concentrations of 0, 0.005, 0.010, 0.015, 0.020, 0.021, 0.022, 0.023, 0.024, 0.025, 0.026, 0.027, 0.028, 0.029, 0.030, 0.032, 0.034, 0.036, 0.038, and 0.040 microgram/ml. It was not possible to distinguish between any two tubes in the range of 0.020-0.040 more than 50% of the time, but in general nearly every analysis sample could be identified with some particular tube and only occasionally with two of standards, suggesting that the over-all precision was about 7%. If the fluorescence was greater than the 0.040 sample, the isobutyl alcohol was diluted to bring it within the range. The standards below 0.020 were seldom used except in determining a very weak blank, where the low precision was unimportant.

The use of pure thiochrome made these high dilution standards possible; standards of quinine or other fluorescence substances could be used only at concentrations of the order of ten to twenty times these standards, and the color differences were always annoying. Good dark adaption of the eyes was necessary for the comparisons, which were made in a photographic darkroom. The assay tubes and standards were viewed in a simple box arranged to illuminate the tubes at an angle of 45° with light from a 2-watt argon glow lamp; the incident light was filtered through a Corning HR Red Ultra glass filter.

100% recovery of added thiamin was obtained under the conditions of the assay over a wide range of added amounts. The materials contributing to the blanks from the urine sample amounted to 0.2-0.4 microgram/ml., estimated as B_1; since the final result

involved the difference of the blank from the sample, the over-all accuracy can be estimated as ranging from 15% for urine concentrations of 0.2 microgram/ml. down to about 8% for the higher concentrations involved in much of the work.

B. Sulfur Analysis and Separation

In line with the objective of determining as much as possible of the over-all behavior in the experiments, it was believed that the form in which any radiosulfur might be excreted would be of importance. The Benedict-Folin (14, 15, 16) fractionation was adopted as representing a reasonable separation into groups and was studied until a method following this general procedure applicable to large quantities of urine was devised. The final procedure was as follows:

Inorganic sulfate: The whole urine (less any small samples for thiochrome analysis, etc.) was diluted to twice its volume and made 0.1 N. in HCl; over a period of hours, without stirring, 1 M. $BaCl_2$ was added very slowly through a fine capillary extending below the surface of the liquid. The solution was then stirred, and the precipitate allowed to settle. The entire supernatant was filtered through Whatman #44 filter paper in a small Hirsch funnel with the aid of gentle suction; the precipitate was transferred to the filter paper; and after thorough washing with cold water (and with dioxane if there seemed to be any appreciable quantities of pigments remaining in the filter paper) the $BaSO_4$ and filter paper were ignited at 850 °C in an electric muffle furnace.

Ethereal sulfate: The filtrate from the inorganic fraction

was evaporated to about one-tenth the original volume on a hot plate at a temperature low enough to prevent undue bumping and yet maintain vigorous boiling. It was diluted to twice its volume and boiled; the precipitate was then filtered from the hot solution, washed with boiling water (and dioxane if necessary), and ignited as above.

Neutral sulfur: The filtrate from the ethereal sulfate precipitate was nearly neutralized, and Benedict reagent was added; the solution was evaporated to about 100 ml., transferred to a casserole, and slowly taken to dryness. The casserole was then ignited to a dull red heat on the hot plate. The residue was taken up in concentrated HCl, boiled, and diluted to about 300 ml.; the $BaSO_4$ was again filtered from the hot solution, as in the case of the ethereal fraction, and ignited.

Feces: Only total sulfur was determined for the feces, the total excretion being taken to dryness with modified Benedict reagent (16) and ignited as usual. $BaCl_2$ was added to the boiling diluted HCl solution of the residue, and the $BaSO_4$ filtered off as in the urine neutral-sulfur analyses.

Check analyses were made on a sample of normal urine until consistent results were obtained and the limits of variation of the various conditions established. Table 3 indicates the results of some preliminary check analyses.

Table 3

Milligrams of $BaSO_4$ Recovered from Urine with and Without Added Sulfur Containing Compounds

Sample	100 ml. sulfur solution[1]	100 ml. urine				100 ml. urine + 10 ml. sulfur solution[1]	
Analysis No.	S-1	A-1	B-2	D-1	E-1	F-1	G-1
Fraction:							
Inorganic	392.2	382.8		384.8		420.4	
Ethereal	51.5	41.7		47.4		50.4	
Inorganic + ethereal	443.7	424.5	426.7	432.2	430.8	470.8	478.9
Neutral	75.6		68.3	64.0	64.4	78.9	78.1
Total	519.3		495.0	496.2	495.2	549.7	557.0

[1]The standard sulfur solution consisted of sodium sulfate, d-camphor sulfonic acid, and cystine, weighed out to provide 391.2, 51.8, and 76.6 mg. of $BaSO_4$ for the inorganic, ethereal, and neutral sulfur fractions, respectively, per 100 ml.

As can be seen from the results with the standard solution, the method is capable of high precision; the considerable difficulty of getting "clean" precipitates without resorting to enormous volumes is probably the main reason for the disagreements of about 1% in over-all recovery. Although high results are to be attributed to general contamination of the weighed $BaSO_4$ precipitates, the later conversion of $BaSO_4$ to elementary sulfur for the radioactive determinations removes possible contamination of the samples; thus the only error would be the quantitative error of the fractionation and recovery. It was believed that less than 0.2% of the total sulfur of the urine escaped recovery and that the fractionation was satisfactory within $\pm$ 2%, the major error being ethereal sulfate that would appear in either the inorganic

or neutral fraction. The errors of the radioactive determinations are of course much greater, as will be seen in Section E, below.

C. Preparation of Samples for Counting

For the preparation of the actual sample to be counted for radioactivity, it had been proposed to metathesize the $BaSO_4$ to Li_2SO_4, since this material would permit a greater amount of activity per unit area of the sample, and it was believed that there would be less absorption from the lithium ion than from the barium ion (17). A procedure for this process had been worked out under the direction of Dr. Yost, as follows:

A 250-mg. sample of the $BaSO_4$ was weighed out and placed together with 25 ml. of 0.2 N Li_2CO_3 in a 125-ml. conical flask equipped with a small reflux condenser. The mixture was boiled vigorously for about 4 hours, cooled, filtered, and washed with 10 ml. of water. The precipitate was washed back into the original flask, and the metathesis repeated twice. All of the filtrates and washings were combined, evaporated to about 15 ml., and washed with 10-15 ml. of hot water. The filtrate was made just acid with conc. HNO_3 and evaporated to dryness in a small vial. The residue was extracted with two 15-20 ml. portions of absolute ethyl alcohol; the alcohol was evaporated, and the Li_2SO_4 dried at 150°C for 2 hours.

The use of this procedure for a few samples demonstrated that it was very time-consuming and required constant attention; other ways of preparing sample material were then considered. The use of elementary sulfur would offer the best possible counting efficiency, and the reduction of the sulfate to sulfide, with reox-

idation to sulfur, seemed an obvious possibility. The reduction of the sulfate with hydrogen was found to be very simple; the $BaSO_4$ samples were heated in silica (or platinum) boats in a silica combustion tube while passing in a slow stream of hydrogen. The extent of the reduction was determined by the weight loss. No appreciable reduction was found after 3 hours at 600-700°C, but 1 hour at 900-1000°C gave the theoretical weight loss for 100% conversion to BaS; if the $BaSO_4$ fused, incomplete reduction occurred.

The oxidation process was accomplished by decomposing the BaS with HCl, and sweeping the H_2S into a KI_3 solution. In the first samples handled by this method the recovery of sulfur was as low as 10%, chiefly because of mechanical losses, but considerable experimentation resulted in the development of the following procedure:

The flask containing the amorphous sulfur and iodine solution was heated below the boiling point for about 1 hour to coagulate the sulfur; finally the sulfur was filtered out on filter paper and the flask and precipitate washed thoroughly with hot water to remove most of the iodine and salts. The filter paper was replaced in the flask, 30 ml. of water added, and the neck was covered with clean parchment paper. The flask was autoclaved at 15 psig for about 10 minutes, by which time all of the sulfur was converted to the rhombic form. The filter paper was pulped and washed onto a sintered-glass filter, the flask and precipitate being washed thoroughly to remove any last traces of salts. The sulfur was then washed out of the flask and filter paper through the filter with liberal quantities of CS_2, with suction to aid in the washing process and evaporate the

filtrate. The sulfur in CS_2 was finally transferred to a weighed 5-ml. beaker and precipitated in extremely fine crystals by adding ether to the nearly saturated solution in CS_2; the ether and CS_2 were evaporated and the sulfur finally weighed. That there was no exchange of sulfur with the sulfur of CS_2 was demonstrated by Cooley (17).

The samples were made into "infinitely thick" films, containing at least 14 mg./sq.cm. of area to provide for a constant self-absorption. The proper area was calculated from the weight of the sulfur, and 1 mg./sq.cm. of cellulose nitrate in amyl acetate was added to sulfur. Amyl acetate was added to form a thick suspension, and the mixture was poured out on a small piece of 0.001-inch-thick aluminum foil cut to the proper area. The beaker was washed with additional amyl acetate if necessary; the suspension on the foil was stirred to distribute the sulfur uniformly, and the solvent allowed to evaporate. The use of the foil cut to the proper size provided a clean edge for the sample; the surface tension of the solvent prevented it from running over. The process produced films which were very uniform right to the edges. The thin foil was mounted on other strips for convenience in handling.

D. Counting Techniques

All of the counting equipment for this work was constructed by Dr. Frank Oppenheimer, under the direction of Dr. D. M. Yost, and incorporated two departures from the usual counting techniques.

One innovation was the use of a counter which is open to the sample, with a single enclosure of both the sample and counter to

contain the discharge atmosphere. This arrangement provides that the maximum amount of radiation from the sample actually reaches the counter, and is of particular value for counting the extremely weak β particles from S_{35} which would be stopped by any but the very thinnest counter envelope.

The second departure is the use of the coincidence method, which has been frequently used in cosmic-ray work and other specialized applications to select the direction or time of the radiation being counted in various ways. The coincidence method is based on the fact that the registering circuits for two (or more) counters can be coupled so that only those counts that are "simultaneous" are registered. With fast, high-resolution counters, the number of spurious or "accidental" coincidence counts is very low; yet any radiation which passes through both counters will be registered. The method offers the possibility of a very low background count with resulting increase in precision per unit time, and the elaborate equipment was constructed to take advantage of this fact. The counters and equipment were unfortunately not useful for coincidence counting; the first attempts at measurements indicated this fact. As a result it was of considerable interest to analyze in some detail the general requirements of a coincidence system, since such an analysis was not available.

1. General analysis of counting methods. In its simplest sense a radioactive determination involves the time rate of a series of random events which are susceptible of statistical analysis (18), and the actual experimental result can be reported with an indication of the precision based on simple statistics. The manner of reporting

this precision is relatively unimportant, since all measures of precision are interrelated, but it should be clearly defined. In this work the probable error was chosen; i.e., it is the error which will be exceeded on the average only 50% of the time. It is convenient to note that twice the probable error will be exceeded only 4.6% of the time.

Whether the coincidence method is of practical use in the counting of routine samples of radioactive material depends on a number of considerations. Thus any radioactive determination is ultimately dependent on the reliability and stability of the equipment used and the competence of the operators, but if it be assumed that all factors such as these are at a high level of reliability, the eventual result of any determination will have a counting error which is dependent only upon the statistical variation of the actual counts; this error can obviously be reduced to any desired value merely by counting long enough. On this basis the somewhat more complex equipment required for coincidence counting can be justified only on the basis of the fact that it offers the possibility of obtaining a smaller error in a shorter time than by counting with a single counter. To make a more precise statement of this it is necessary to do some algebra.

In most cases the numerical result of a particular radioactive determination is expressed simply in terms of the counts for some particular time interval, e.g., t seconds. The probable error of this measurement would then be given by PE = $0.6745\sqrt{n}$, the counting actually being carried out for t seconds and n counts being observed. Suppose the count were made for some T number of t unit

periods. Then $N = nT$ counts would have been observed, and the probable error for the counts per t unit would be $0.6745\sqrt{n/T}$. In the general case the background of the particular counter must be taken into consideration; there are then two counts, and the desired quantity is their difference. The number of counts due to the sample, which is defined as $c = n - b$, where n is the number of counts per unit t period observed when the sample is being counted, and b the counts with all other conditions identical except that the sample is not present. By the usual methods of error combination it can be written that $PE_c = 0.6745(n/T_n + b/T_b)^{1/2}$, T_n being the number of time units the sample has been counted and T_b the number of time units the background has been counted. The total time $T = T_n + T_b$ which has been required for all of the counting can be minimized by appropriate adjustment of the ratio T_n/T_b; for any fixed time T it is possible to choose this ratio so as to give the smallest value to the error of the counts of the sample. By substituting $T_b = T - T_n$, differentiating, and setting the differential equal to zero it is found that $T_n/T_b = (n/b)^{1/2}$ is the condition for which PE_c will be a minimum. If this criterion for the most efficient use of counting time is substituted in the usual error expression, there is obtained $PE_c = 0.6745\ (n^{1/2} + b^{1/2})/T^{1/2}$. Since in the usual case the interest is not so much in the absolute value of the error as in its value relative to the actual number of counts, it is convenient to define a "fractional error" as $f_x = e/x$, where the error e may be whatever measure of the dispersion is being used, and x is the quantity. Taking the "fractional probable

error" = f = PE_c/c, we have at once:

$$f = 0.6745 \frac{n^{1/2} + b^{1/2}}{cT^{1/2}} = 0.6745 \frac{(c+b)^{1/2} + b^{1/2}}{cT^{1/2}} \qquad (1)$$

It is now possible to specify the criterion for the use of coincidence counting more precisely; the coincidence method can be justified only if $f_c < f_s$, where these are the fractional errors of the coincidence and single count, respectively, for the same total time T. The analysis can be extended to include the geometry of the counting arrangement fairly readily by considering an arrangement of two cylindrical counters in a two-dimensional case, neglecting any end effects. Any coincidence method requires two counters, and the same particles must be counted by both; in making an analysis of the method it is convenient to consider the first of the two counters as representing a single-counter system and to contrast the errors involved in using this counter alone with the errors involved in the coincidence counts from the pair.

The error using this first single counter alone will depend on the number of particles from the sample passing through it and its efficiency for the particular radiation; it is obviously desirable to make this counter as large as possible and put the sample as close to it as practical. Now in the ideal case all of the counts from the sample registering in the first counter will also register in the second; in such an ideal case the coincidence method would certainly be justified, as can be seen from Eq. (1). The fractional error will be decreased by any decrease in background, and the sample counts c are identical; certainly there must be some (at least one particle!) of the background radiation counted by the single counter which do

not produce a coincidence count. But this ideal case cannot exist. The first counter was made as large as possible; for all of the radiation from the sample which has entered the first also to enter the second requires that the second counter be larger.

To sum up, there must be, for one reason or another, less coincidence counts than single counts in the first counter. To permit a general treatment, let $\alpha = c_c/c_s$, or the ratio of coincidence counts to single counts for some particular arrangement; the maximum value for α will be fixed by the geometry involved. Also let $\beta_c = c_c/b_c$, and $\beta_s = c_s/b_s$, where the subscripts refer to the coincidence or single counts, respectively. Equation (1) may now be written $f_c = 0.6745\ (1 + \sqrt{1+\beta_c})/\sqrt{c_c T \beta_c}$ and similarly for f_s with the appropriate subscripts. After some rearrangement the requirement of the criterion that $f_c < f_s$ can be written

$$\frac{1 + \sqrt{1 + \beta_c}}{\sqrt{c_c \beta_c}} < \frac{1 + \sqrt{1 + \beta_s}}{\sqrt{c_s \beta_s}} \qquad (2)$$

and if $R = b_c/b_s$, this expression can be eventually transformed to

$$2\sqrt{R} < \alpha(1 + \sqrt{1+\beta_s}) - \beta_s/(1 + \sqrt{1+\beta_s}) \qquad (3)$$

as the relation between R, α, and β_s that must hold for the error of the coincidence counts to be less than that of the single counts (for the same length of total time of counting) with the background and sample count times apportioned for the most efficient use of the total time in each case.

Clearly a limiting value for R is zero (the background of the coincidence counts can hardly be less than zero), and the maximum value for R is of course 1, representing the unlikely event that the coincidence arrangement does not decrease the background

counts per unit time at all. It might be noted that when β_s is very much less than 1 (the case for extremely weak samples), the expression reduces to $R<\alpha^2$. The nature of the relationship between the three parameters is most clearly seen with the aid of Fig. 6, where α and β_s are plotted for a series of selected R values. In interpreting this figure, it should be noted that, for a sample of given activity which has then a particular value of β_s or number of counts in relation to the background of the single counter, and for a particular geometry of the coincidence arrangement given by α, the values of R are the limiting maximum values for which the coincidence arrangement might be useful. If this particular coincidence arrangement does not reduce the background below the indicated R value, it is less efficient than counting with the first (or single) counter alone.

Figure 6 also discloses most clearly another interesting point. Even if the coincidence method should reduce the background counts to 0, there are still severe limitations on the permissible values of α. Thus even for a very strong sample (e.g., with $\beta_s = 100$), α must be greater than 0.82; for a weak sample, with $\beta_s = 1$ (e.g., the counts from the sample are just equal to the usual background of the single counter), α still must be greater than 0.18 (e.g., at least 18% of the particles detected by first single counter must also register in the coincidence counter).

It should not be difficult to construct a satisfactory coincidence system for use with weak samples, and in the counting of many routine samples such a system might offer a considerable saving in time. The ratio of the times involved in counting to the

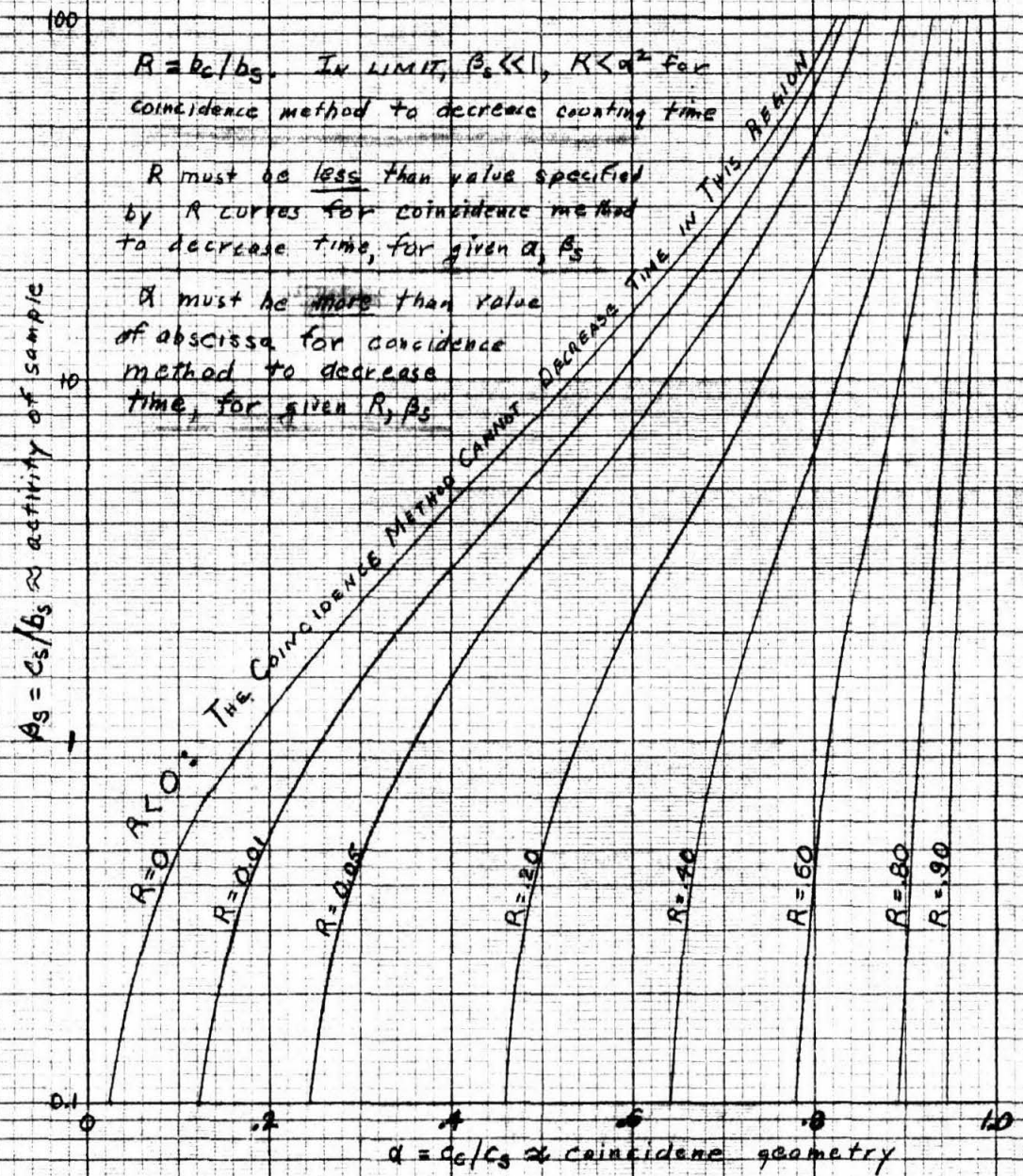


FIG. 6 Relation between sample strength, coincidence geometry, and reduction of background for which the coincidence counting method would increase the precision for a fixed time of counting.

same fractional error will of course be $T_c/T_s = R/\alpha^2$, when β_s and β_c are small in comparison with 1. For such samples, if the coincidence system reduced the background to 1/20 (R = 0.05), which should be possible with fast counters, and if the geometry is 1/3 (α = 1/3), which should be possible with high efficiency counters, then T_c/T_s = 0.45. The time required with such a coincidence system would less than half of that when a single counter is used.

2. Counters. The two counters were made of copper, 1 inch in diameter and 4 inch long, and were coated with sulfide by dipping in a solution of sodium sulfide. Later it was found that $(NH_4)_2S$ made by Swift's method (19) was apparently much better than the usual sodium sulfide solutions. The first counter had two axial slits, 3/8 inch wide, along the front and back; the second counter had such a slit along the front only. The back slit of the first counter was covered with two thicknesses of 0.3-mil-thick aluminum foil to stop photoelectric propagation between the counters. The two counters were mounted, with the sample fixed in front of them, under a large bell jar which could be evacuated and filled with a 30% alcohol and air mixture to a total pressure of 50 mm. of Hg. These counters did not have very flat plateaus, and reproducible results from one filling to another were impossible; accordingly a simple belt arrangement on which the samples were mounted was constructed. It could be operated from outside the bell jar, and hence different samples, or the background with no sample, could be counted under identical conditions. Figure 7 is a sketch of the bell jar containing the arrangement which was used through the course of the work.

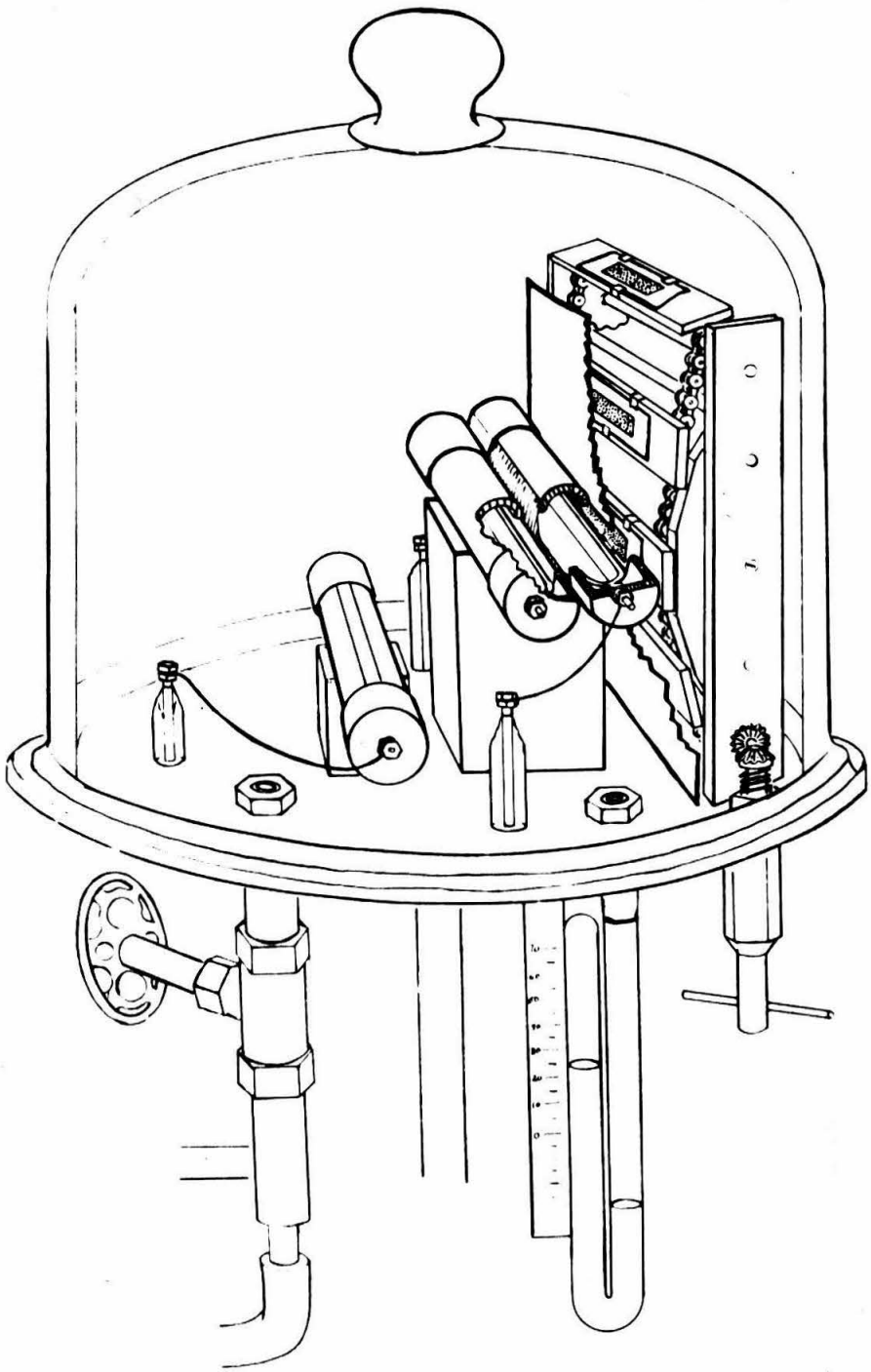


FIG. 7

The geometry of the coincidence arrangement is shown in Fig. 8. In the preparation of the samples it was considered best to utilize all of the available material and prepare the samples of different size. The width of the samples was usually about 9 mm., and the length was varied to provide the proper area for the desired density of 14 mg./sq.cm. The variations of course make for slight differences between various samples but can readily be corrected for, and all the measurements were, in the final calculations, converted to point-source values. The effect of the area variations was treated as follows:

Referring to Fig. 9, we consider the two-dimensional case of a counter 2a long, with a sample 2k long, with the center of the sample at the center of the counter. At any point x, measured from the center line, only a fraction f_x of the particles can enter the counter, even though particles are being emitted in all directions. This fraction in the horizontal plane will be of course $(A_x + B_x)/n$, where n is the average number per unit time, and the angles A and B are measured from the point x. The average fraction from the center line to the end of the sample will be $\frac{1}{nk}\int_0^k (A_x+B_x)dx$, which can be written in terms of the distances a, x, and d and finally integrated to give as the average fraction F_k

$$F_k = \frac{1}{nk}\,(a+k)\,\tan^{-1}\frac{(a+k)}{d} - (a-k)\,\tan^{-1}\frac{(a-k)}{d} - \frac{d}{2}\ln\frac{d^2+(a+k)^2}{d^2+(a-k)^2}$$

It is now possible to take the ratio of this average fraction to the fraction for a zero-length sample and obtain a length factor L, which is the number by which the observed counts must be multiplied

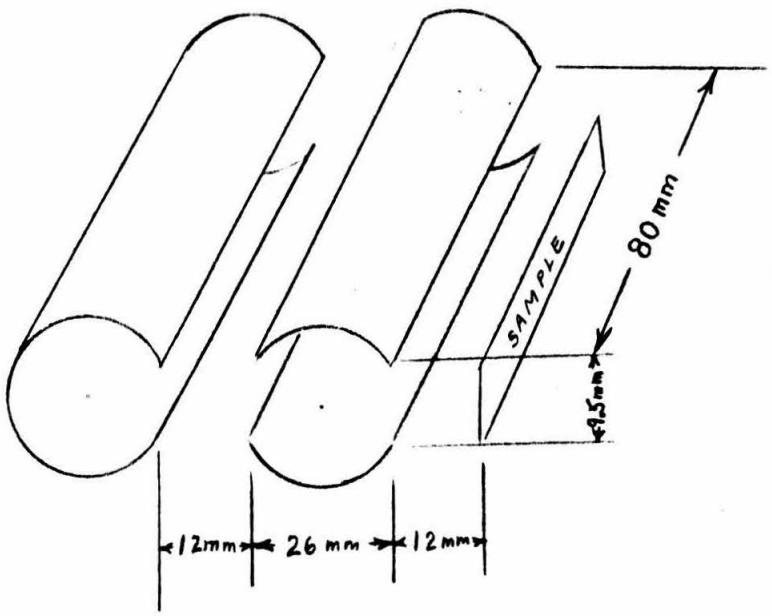


FIG. 8. GEOMETRY OF THE COUNTERS USED

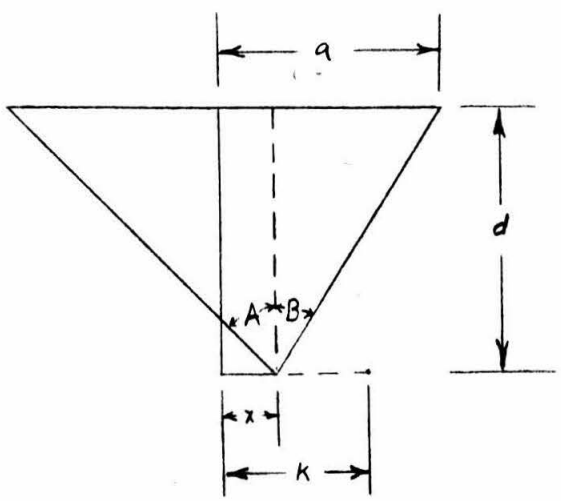


FIG. 9. DIAGRAM ILLUSTRATING SYMBOLS USED IN CALCULATION OF AREA EFFECTS

to give the number of counts which would have been obtained if the sample had been of zero length. A similar treatment was made for variations in sample width. Tables 4 and 5 give these factors for various lengths and widths for which the calculations were made on the basis of the counter geometry of Fig. 8. The factors used in the calculations were taken from graphs of the data in these tables.

Table 4

Horizontal Fraction of the Total Radiation Reaching the Counters

Sample length mm.	Fraction reaching		Length factors	
	Singles counter	Coincidence counter	L_s	L_c
0	0.8145	0.4296	1.0000	1.0000
4	0.8140	0.4295	1.0006	1.0002
10	0.8136	0.4289	1.0012	1.0017
20	0.8110	0.4270	1.0041	1.0061
40	0.7993	0.4196	1.0190	1.0241
60	0.7740	0.4076	1.0523	1.0540
70	0.7526	0.4001	1.0824	1.0737
80	0.7229	0.3918	1.1266	1.0954

Table 5

Vertical Fraction of the Total Radiation Reaching the Counters

Sample width mm.	Fraction reaching		Width factors	
	Singles counter	Coincidence counter	W_s	W_c
0	0.2399	0.06030	1.0000	1.0000
2.5	0.2393	0.06028	1.0028	1.0002
4.5	0.2378		1.0092	
8.0	0.2325	0.06016	1.0318	1.0023
9.0	0.2316	0.06014	1.0362	1.0027
9.5	0.2307	0.06012	1.0402	1.0029

It is to be noted that the geometry of the coincidence system can be directly obtained from the fractions of these tables for any particular sample, but it is clear that the fractions do

not vary particularly with sample size. Thus for a zero - size sample, $\alpha = 0.4296 \times 0.06030/0.8145 \times 0.2399 = 0.132$, and for the largest sample used, $\alpha = 0.3918 \times 0.06012/0.7229 \times 0.2307 = 0.141$. Referring to Fig. 6, it can be seen that for a geometry with $\alpha = 0.14$, even if the coincidence background were reduced to zero ($R = 0$), this coincidence system would be less efficient than using the first single count for any sample except those with $\beta_s = 0.7$, i.e., those so weak that the total counts were only about 70% of the singles background. The actual experimental fact was that for the system as originally devised R was greater than 0.1; therefore this coincidence system, as constructed, was less efficient for any sample of any activity than was the use of the first single counter alone.

3. Electronic equipment. The electronic equipment initially included six amplifiers, each with a 57 tube in a Neher-Harper quench circuit, followed by appropriate amplification, pulse clipping, a Rossi coincidence circuit, and the usual output amplification. Each amplifier could count either the single counts of the particular counter tube to which it was connected or the coincidence counts between this particular counter and any other connected to the coincidence circuit. Four scale-of-eight counting and recording units were provided, together with a power supply for the amplifier and a high-voltage supply for the counters.

In the early stages of the research the eccentricities of this equipment rather seriously interfered with the experimental work. It was found necessary to select particular samples from among those prepared and to concentrate on counting them; by the

time these were counted, the remaining samples were so weak that they could not be counted; only a fortunate choice of samples provided the essential information. During this period the equipment operated satisfactorily about 25% of the time; this low efficiency was primarily a result of marginal design, with failure of inadequate components and poor soldering as contributing factors. Most of the effort was concentrated on simple repair and replacement as a result of failure during this time, with the emphasis on counting as many samples as possible. From time to time there were serious discrepancies between the single and coincidence-counting results far beyond any statistical variation; in cases such as this the results were discarded and the counting was repeated. The fact that there were more amplifiers and scaling units than were actually required at any one time was a help, since a spare was sometimes available when serious trouble occurred. However, it was also a handicap, since the temptation to record the same counts simultaneously on two different recorders was very strong; when the two recorders gave different results, it was necessary to discard both results, since there was no way of determining which was correct.

E. Results

1. Calculations. In the determination of radiosulfur all of the measurements involve the comparison of the activity (as determined from the counting rate) of some particular sample with the activity of a standard prepared from and containing a known amount of the same radioactive thiamin. Consequently the results can be directly expressed in terms of the micrograms of

thiamin. It was necessary to multiply the observed activities by the appropriate L and W factors in order to convert all samples to a standard zero-length, zero-width basis, and it was found convenient to include also the F and T factors. Very few of the samples represented all of the theoretical sulfur of either the original material or the $BaSO_4$ precipitate from which it was derived, and this fraction was denoted by F, the ratio of the total sulfur (from the original material) in the sample to the theoretical sulfur of the original material involved. In some cases the samples were made utilizing all of the available sulfur and were greater than 14 mg./sq.cm. thick. Since less than 0.1% of the radiation from below the top 14 mg. could reach the counters, the contribution of this material to the observed counts was considered negligible, and the T factor was defined as the thickness (in mg./sq.cm.) of the actual sample divided by 14. Thus the final expression used was $y_1 = (y_2/L_2W_2T_2)(L_1W_1T_1/F_1)R_2^1$, where R_2^1 is the ratio of the counts above background for the sample 1 to the counts above background for the standard 2, and y_1 gives the micrograms of thiamin in the original material 1 for the standard sample 2 containing y_2 micrograms of thiamin.

2. Standard samples. Some six standard samples were prepared from the original radio-B_1 solution for use in the counting work. The first two were prepared without any added carrier material, and the $BaSO_4$ obtained was metathesized to Li_2SO_4. Considerable difficulties were encountered, and these samples did not agree with each other. Only a few of the excretion samples were metathesized to Li_2SO_4 and compared with the first of these samples (S-1).

This standard was later calibrated by comparison with the better standards, and this calibration was used for the calculation of the five excretion samples of the Li_2SO_4 type that had been measured against it. One of these Li_2SO_4 excretion samples was later converted to elementary sulfur and again counted against an elementary sulfur standard; the agreement with the Li_2SO_4 value (based on the calibrated S-1 standard) was within the experimental error.

In all of the remaining four standards inert carrier was added; in two cases a fractionation was carried out to determine whether any of the radiosulfur in active thiamin could appear in the inorganic or ethereal fractions as a result of exchange. The analytical details of these samples and the details of their intercomparison are given in Tables 6 and 7. No evidence of exchange was detected, and the agreement between the standards was excellent.

Table 6

Details of Preparation of Standards

Standard No.	S-1	S-3i$_1$	S-3i$_2$	S-3n	S-4i	S-4e	S-4n	S-5	S-6
Thiamin taken, mg.	15.8			1.58			5.3	5.3	5.3
Carrier material added		25 ml. S solution[1] 144.7 mg. cystine			400 ml. urine			4 ml. of S solution[1]	1.9 ml.
Theoretical $BaSO_4$, mg.	11.0	975.5		601.4				211.4	101.4
Recovered $BaSO_4$, mg.	11.0	977.4		557.2	4914.0	351.8	495.1	114.5	101.2
Converted to	Li_2SO_4	S		S	S	S	S	S	S
Recovered, mg.	8.2	53.5	27.3	44.2	222.6	24.8	32.4	11.0	7.5
Fraction of thiamin = F	1.0	0.399	0.203	0.540	0.335	0.514	0.416	0.378	0.539
Inert material added, mg.	12.5							43.4	68.4
Total sample material, mg.	20.7	53.5	27.3	44.2	222.6	24.8	32.4	54.4	75.9
Sample width, mm.	9.5	9.5	9.0	9.0	9.5	8.0	9.0	9.5	9.5
Sample length, mm.	16.0	40.0	21.6	35.0	90.0	22.3	25.7	40.8	49.5
Single factor=L_sW_sT/F	1.014	1.086	1.052	1.053	2.269	1.037	1.015	1.062	1.136
Coincidence factor=L_cW_cT/F	0.978	1.037	1.012	1.024	2.093	1.011	0.985	1.030	1.195
Thiamin content, based on analytical data	15,800			855			2410	1980	2280
activity vs S-6							2340 ± 35	2130 ±110	
activity vs S-4				816 ±35					
activity vs S-3	7000 ±240	10±14	35±87		−240 ±136	81±26			
Final value used	7000			855			2410		2280

[1]The composition of the standard sulfur solution is given in Table 3, p. 18.

Table 7

Intercomparison of Standards

Samples Compared	Ratios of activities	
	Singles	Coincidences
S-4n/S-6	0.917 ± 0.0147	1.125 ± 0.05
S-5/S-6	0.819 ± 0.046	0.805 ± 0.13
S-3n/S-4n	0.315 ± 0.015	0.412 ± 0.04
S-2/S-1	0.103 ± 0.004	0.091 ± 0.008
S-1/S-3	8.5 ± 0.8	
$S-3i_1/S-1$	0.0006 ± 0.0008	0.0004 ± 0.002
$S-3i_2/S-3$		0.0085 ± 0.06
S-4i/S-3	-0.0175 ± 0.0085	0.0207 ± 0.0054
S-4e/S-3	0.0207 ± 0.005	-0.0586 ± 0.027

3. Data obtained. The counting process consisted of placing a series of samples and a standard on the plate carrier in the bell jar, leaving at least one plate blank for a background, and alternately counting the background, sample, and standard until enough counts had been obtained to assure the desired precision. The samples and background were alternated over various short-period times, and these short-period counts were checked for normal statistical behavior; any series in which there were serious discrepancies (and there were many at first) were discarded and the samples rerun. It was eventually possible to count for 3 or 4 days without any drift in the background or at most with a slow linear change with time. In the latter case appropriate weighted averages of the short-period counts were taken, but in many cases the entire series of counts could merely be totaled. Table 8 contains the analytical data for two typical samples, and Table 9, the counting data for these two samples. Tables 10 and 11 contain the complete final summary of all samples measured, as well as the thiochrome assay values.

Table 8

Analytical Data for Two Typical Samples; B_1-Free Diet

Sample No.[1]	1-17i	1-12n
Fraction of total urine taken	0.981	0.990
$BaSO_4$ recovered, mg.	1885.7	474.4
Sulfur to sample, mg.	108.5	33.1
Fractional content = F	0.413	0.509
Sample width, mm.	9.5	9.0
Sample length, mm.	77.5	26.3
Singles factor = $L_s W_s T/F$	2.65	2.04
Coincidence factor = $L_c W_c T/F$	2.56	1.99
Singles: activity/S-1	0.0120 ± 0.0004	
activity/S-3		0.279 ± 0.014
B_1 content, micrograms	218 ± 7	463 ± 24
Coincidences: activity/S-1	0.0052 ± 0.0049	
activity/S-3		0.269 ± 0.032
B_1 content, micrograms	37 ± 31	446 ± 53
Average of singles and coincidences, micrograms	192 ± 6	461 ± 22

[1]Sample numbers represent the date (month and day) with a lower case i, e, or n to indicate the inorganic, ethereal, or neutral urine fractions, respectively, and a capital F for the total sulfur from feces. Table 10 and Fig. 10 show these two samples in relation to the other data.

Table 9

Counting Data for Two Typical Samples; B_1-Free Diet

Sample	Time seconds	Counts		Counting rate/1000 seconds	
		Singles	Coincidences	Singles	Coincidences
1-17i	154,082	168,667	13,515	1094.7 ± 1.8	87.71 ± 0.7
Background	148,600	154,515	12,704	1039.8 ± 1.7	85.52 ± 0.7
S-1	10,000	50,619	5,052	5062 ± 23	505 ± 7
1-17i − Background				54.5 ± 2.5	2.19 ± 0.9
S-1 − Background				465 ± 24	420 ± 8
Ratio: 1-17i/S-1				0.0120 ± 0.0004	0.00521 ± 0.0025
1-12n	41,900	57,043	5,038	1361.4 ± 3.8	120.24 ± 1.2
Background	155,300	196,948	16,956	1268.2 ± 1.9	109.18 ± 0.6
S-3n	16,400	26,280	2,489	1602.4 ± 6.6	151.77 ± 0.2
1-12n − Background				93 ± 4	11.1 ± 1.3
S-3n − Background				334 ± 7	42.6 ± 2
Ratio: 1-12n/S-3n				0.279 ± 0.014	0.269 ± 0.032

Table 10

Data from B_1-Free Diet Experiment

All quantities in micrograms of thiamin

Date	Injected	Urinary Excretion			
		Free B_1 (thiochrome assay)	Radioactive sulfur		
			Inorganic	Ethereal	Neutral
12-1	B_1-free diet begun				
12-31	0	0			
1-2	0	0			
1-3	0	0			
1-4	2640	70			
1-5	2640	450			−143 ± 132
1-6	2640	510	200 ± 93	390 ± 200	
1-7	2640	480	145 ± 42	850 ± 135	475 ± 50
1-8	2640				
1-9	2640	350		−100 ± 43[1]	
1-10	2640	330		−100 ± 43[1]	
1-11	2640				625 ± 24
1-12	2640		85 ± 32		461 ± 22
1-13	2640	390	40 ± 22	51 ± 7	352 ± 44
1-14	0	90			
1-15	0	70			
1-16	0	36		−117 ± 134	
1-17	0	0	192 ± 6	56 ± 7	−61 ± 18

[1]The urine for 1-9 and 1-10 were combined. The numbers given for each day are half the quantity observed for the combined sample. The thiochrome assays were made before combining.

Table 11

Data from Normal Diet Experiment

All quantities in micrograms of thiamin

Date	Injected	Urinary Excretion				Feces
		Free B_1 (thiochrome)	Radioactive sulfur			Total S*
			Inorganic	Ethereal	Neutral	
7-10	0	350				
7-11	0	520				
7-14	0	400				
7-18	0	450				
7-19	15800	8900			950 ± 200	−90 ± 31
7-20	15800	8700	755 ± 175	−62 ± 28	6160 ± 73	−284 ± 198
7-21	15800	9400		154 ± 32	6240 ± 157	192 ± 28
7-22	15800	11500		6640 ± 276[1]		774 ± 41
7-23	0	1950		5710 ± 88[1]		1500 ± 44
7-24	0	520		−205 ± 210	1650 ± 96	1380 ± 80
7-25	0			61 ± 13	1770 ± 95	174 ± 60[2]
7-26	0	480	400 ± 250		711 ± 115	2020 ± 25
7-27	0	450				481 ± 48
7-28	0		3690 ± 129	−390 ± 195	1110 ± 58	192 ± 20

[1]The ethereal and neutral fractions were combined in the analysis; the graph Fig. 11 assumes this is all neutral sulfur.
[2]Only a very small quantity of feces in this period; the graph Fig. 11 uses the average of the excretion for 7-25 and 7-26 for each day.

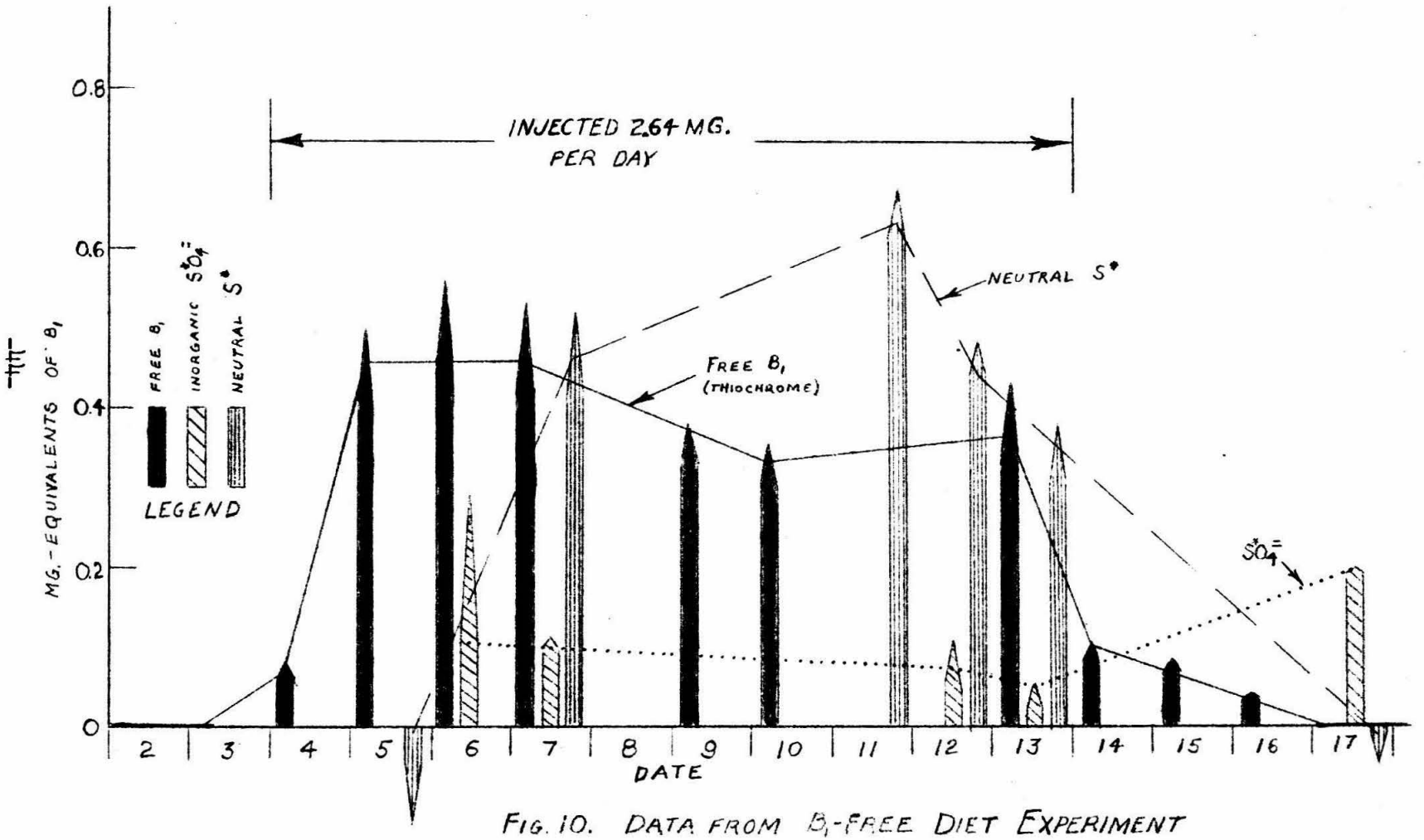


FIG. 10. DATA FROM B_1-FREE DIET EXPERIMENT

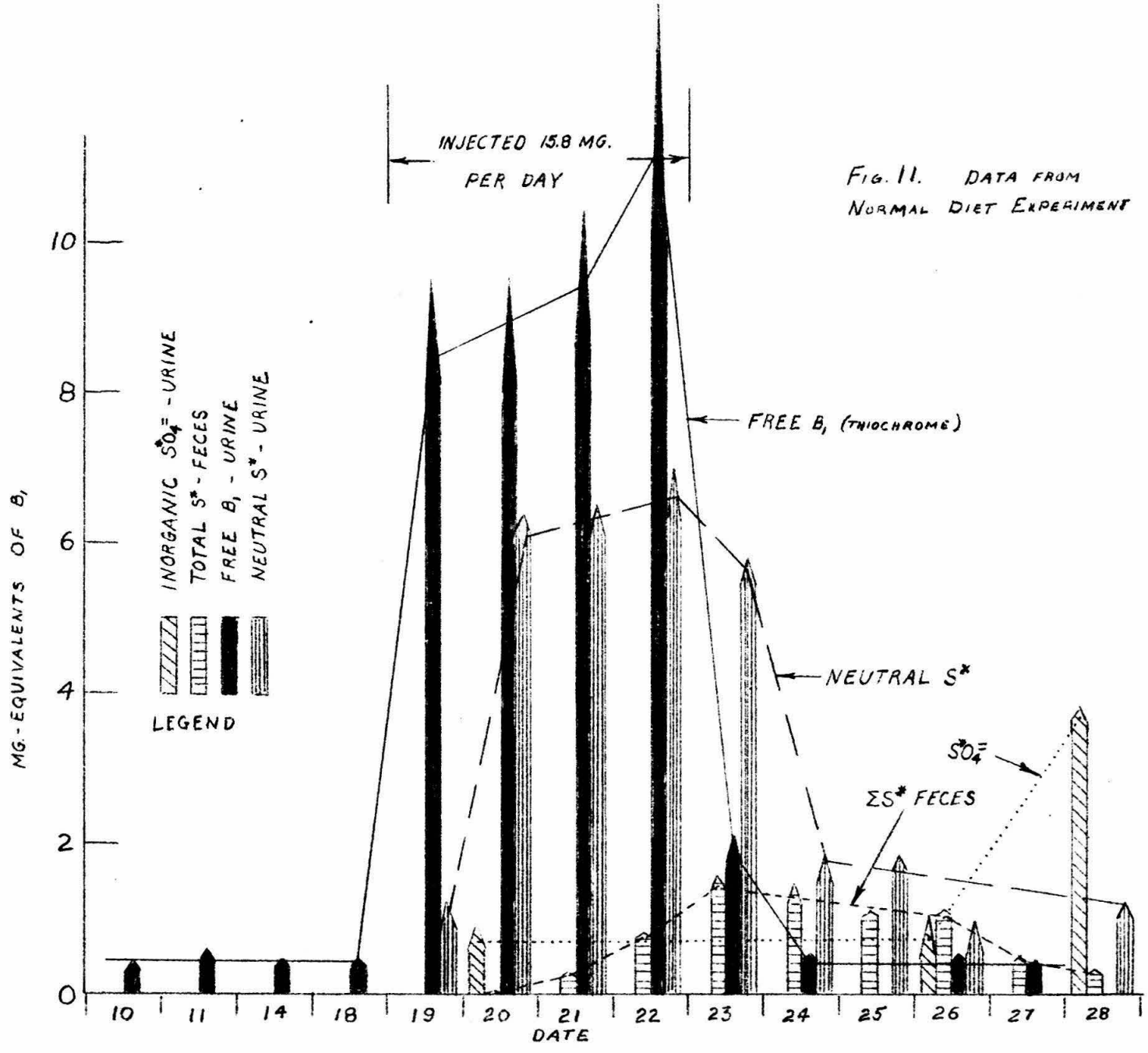


FIG. 11. DATA FROM NORMAL DIET EXPERIMENT

4. Discussion. The essential features and conclusions from this research are fully covered in Part II, but there is one point of a more speculative nature which might be noted.

Only 84% of the total administered radiosulfur was recovered in the normal-diet experiment: 10.6% in the feces and 73.4% in the urine; 13.3% as inorganic sulfate, 0.6% as ethereal sulfate, and 59.5% as neutral sulfur. The rate of excretion by all four paths is decreasing from all except the inorganic sulfate, which rises as the experiment was discontinued in this as well as in the B_1-free diet experiment. This increase suggests that the remainder of the radiosulfur would be excreted as inorganic sulfate within the next few days.

The mechanism may be similar to that of the experiment (20) in which radioactive sulfur in the form of Na_2SO_4 was ingested. In this case only about half the radiosulfur appeared in the first day's urine and at roughly a constant rate; instead of a continuing and decreasing excretion, as might be expected from simple dilution within the body, none could be detected within the next 2 days. A possible inference is that the remainder of the ingested inorganic sulfate was "bound" in some manner and would eventually appear only after considerable delay, perhaps as the result of catabolic processes.

If this notion is applicable, then the inorganic sulfate portion of the excreted radiosulfur in the thiamin experiments may represent only half of the actual amount which is produced as a result of the destruction of the radioactive thiamin. This possibility

leads to the conclusion that there exists within the body, at the end of the experiments, another 13% of the total ingested amount as inorganic sulfate; this amount, added to the total experimental recovery, accounts for 97% of the total injected; a recovery which is well within the experimental error. The rise in inorganic sulfate excretion at the end of the experiments might well be the beginning of the excretion of this "bound" material.

It is interesting that the quantity of inorganic sulfate appearing during and following the injections before the final increase is of the order of 4-5% of the daily injected quantities in both cases, apparently independent of the body reserves. This result may be compared with the approximately 6% destruction observed in the rat (9, 10) at high-level intakes.

IV. MISCELLANEOUS CONTRIBUTIONS

The low activity of the sulfur samples which were measured in the course of this research was an obvious major difficulty, and considerable thought was given to various solutions of this problem. The preparation of sulfur of greater specific activity would be one possibility, and one possibility considered was the construction of a Clusius diffusion column to make an enriched S^{34} sulfur sample for the cyclotron irradiation. No work was done on this problem beyond consideration of the preliminary design of a 30-foot column to operate with H_2S and give a fivefold enrichment; the indications were that it would be relatively easy to set up and operate, and the tentative calculations gave 2 months as the operating time to provide sufficient sulfur for an irradiation sample similar to the one which had been used.

A. New Synthesis of the Vitamin B_1 Thiazole

When the details of the actual preparation of the thiamin from the sulfur were considered, it was believed that the synthesis itself offered considerable promise as a means of obtaining samples of higher specific activity, merely because such high dilutions had been used in preparing the sample used in this work. The scale of the synthesis carried out by Dr. Buchman's students involved starting with 1 g. of sulfur, and the small bombardment sample was diluted to this amount before the synthesis was started. The method used, which involved the preparation of the thiazole from thioformamide

(prepared from P_2S_5), had produced an actual yield of only 8% based on the sulfur used. Thus it seemed clear that there might be possibilities of improvement in this particular synthesis by experimental work directed at obtaining high yields based on the sulfur rather than on the other ingredients. The question as to whether there were any alternative syntheses of the thiazole portion of thiamin seemed also pertinent.

An extensive literature search and much paper chemistry led to one alternative that was attractive because of its simplicity; it involved proceeding via the general steps S → H_2S → thiourea → amino-thiazole → thiazole. The key to this sequence was apparently the possibility of removal of the amino group; that it be diazotized and reduced was considered very unlikely in view of the previous work that had yielded almost no success using this type of reaction with a wide variety of amino-thiazoles.

Accordingly some experimental work was undertaken to demonstrate whether this particular synthesis would be feasible and deserving of further study. The first step, the conversion of the sulfur to H_2S, seemed to require only minor study to develop a simple apparatus and technique for handling the small quantities that would be involved with no loss, and it was omitted. The second step seemed simple enough and offered the possibility of high yields for the preparation of thiourea from H_2S, particularly in view of a procedure provided in a personal communication (21) which gave 87% yields, based on cyanamide, and personal discussions suggesting that the yield based on sulfur should be equally as good if precautions to conserve the H_2S were taken. The procedure as received is

recorded here:

> A mole of solid cyanamide was diluted with water to the point where the dicyanamide present as impurity was 2% (17-27%). This solution was placed in tight (gas) vessel which may be a 3-neck flask fitted with a gas-tight agitator, thermometer and gas inlet tubes through rubber stoppers which have been wired down. The mixture was treated with H_2S under a positive pressure up to 15 lbs. for 15 min. The pressure is released and 0.55 equivalent of aqueous 27% NH_3 is added. The pressure is resumed and the mixture heated to 40° C for three hours at constant pressure. The conversion of cyanamide to thiourea was 98%. The pressure was released and the solution cooled to 25° C and the first crop filtered off and washed with small amount of water (cold). These crystals are 98% pure or better. The filtrate is neutralized with HCl acid and then a further quantity of HCl (9 cc./100cc) solution added.This solution was boiled for 15 min. to convert the dicyanamide to soluble guanylurea hydrochloride. Neutralize with concentrated NH_3. The solution was vacuum evaporated to 1/3 its volume and cooled to 25° C to give the second crop which was 81% pure and after once recrystallization from water was 98% pure or better.
>
> The yields were 81% for the first crop; 6.5% for the recrystallized second crop; over all yield 87.5%; m.p. 175 (Dennis Melting Point Bar.) Side reactions: dicyandiamide and urea - small quantity - probably 2% of the cyanamide. Guanyl thiourea - unlikely - reaction of H_2S and dicy very slow.

The experimental work was begun at the third step, first using CH_3COCH_2Cl and thereby making 2-amino-4-methyl thiazole essentially as described in the literature (22) but with modifications to obtain high yields based on the thiourea used. When trials of removing the amino group by diazotizing with $NaNO_2$ in HCl followed by reduction with hypophosphorous acid seemed successful, later attention was directed at the vitamin thiazole itself.

Four batches of the thiamin thiazole, 2-amino-4-methyl-5-β-hydroxy-ethyl thiazole were prepared from thiourea and $(CH_3COCHClCH_3CH_2O)_2O$ (the anhydride of 3-chloro-5-hydroxyl-2-pentanone-chloro-aceto-propyl ether), which was provided

through the courtesy of Dr. Buchman. The yield, based on thiourea, was improved on each trial, as increasing excesses of the ether anhydride were used, and the last gave something greater than 78%, as follows:

3.8 g. of thiourea dissolved in about 150 ml. of water was heated to boiling under a reflux condenser; 9.52 g. of the ether was run in slowly from a separatory funnel and washed in with absolute EtOH. 12 ml. of concentrated HCl was added, and the solution evaporated under a vacuum, dissolved in absolute EtOH, and finally evaporated to 80 ml. On the addition of 300 ml. of ethyl ether, 9.01 g. (representing the 78% yield) of the amino dihydrochloride separated out. On the basis of the other trials, another 5-10% should have been obtained by another crystallization.

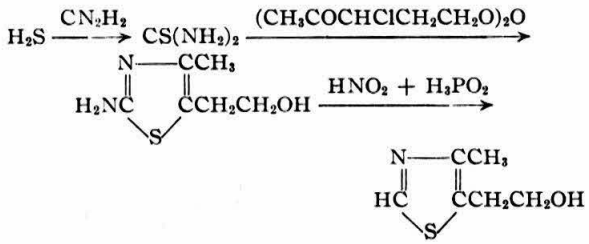
The removal of the amino group was quite successful and gave a yield of 31% on the first trial. This work was confirmed and improved by others (23), and the details of the experiment have been published:

[Reprinted from the Journal of the American Chemical Society, **69**, 465 (1947).]

The Reduction of 2-Amino-4-methyl-5-β-hydroxyethylthiazole in the Synthesis of Thiamin

By J. B. Hatcher[1]

In the course of studies involving the use of thiamin labelled with radioactive sulfur[2] the low yields, as based on sulfur, of the usual synthesis resulted in samples of the vitamin very weak in radioactivity. It is felt desirable to record the preliminary work, discontinued at the beginning of the war, which indicated the feasibility of an alternative synthesis for the thiazole portion of the vitamin. The steps are



Experimental

The first step was not studied, but should offer no particular difficulties in obtaining high yields since there are no significant side reactions. The second step was found to give yields of 80% when an excess of the acetopropyl ether was slowly run into an aqueous solution of thiourea at 100°. The third step is essentially new, since the literature records an impressive list of failures with this type reaction with various amino thiazoles. However, the first trials of the reaction step above gave good yields, *e. g.*, 31%, as follows: 2 g. of the aminothiazole dihydrochloride was dissolved in about 15 ml. of 12 *N* hydrochloric acid in a 50-ml. Erlenmeyer flask and cooled to 0° by swirling in an ice-bath. An equivalent amount (4.1 ml.) of 2 *M*

(1) Present address: The Argonne National Laboratory, Chicago, Illinois.

(2) Buchman, Hatcher, Yost, and McMillan, *Proc. Natl. Acad. Sci.*, **26**, 412 (1940).

sodium nitrate solution was run in slowly drop by drop with vigorous shaking and swirling of the flask. Fifteen ml. of 30–32% H_3PO_2 was then added slowly, with the flask still in the ice-bath, and finally the mixture was allowed to warm to room temperature. It was then made alkaline with 6 *N* sodium hydroxide and diluted to about 1 liter, and extracted with five 10-ml. portions of ethyl ether. The ether extracts were combined, evaporated on a hot-plate to about 10 ml. and transferred to a distilling flask. The remainder of the ether was removed under vacuum at room temperature, and the residue vacuum distilled at 120–130° giving 0.38 g. of a colorless liquid. This liquid gave a picrate melting at 162° (uncor.) and the picrate gave no depression of the melting point when mixed with a sample of the picrate prepared from the pure thiamin thiazole prepared by the usual methods.

On the basis of these results it was concluded that the proposed synthesis would offer considerable advantages in the preparation of vitamin B_1 for the purposes, and that the removal of amino groups from thiazoles by diazotization and reduction is by no means as difficult as the literature indicates.

PASADENA, CALIFORNIA RECEIVED AUGUST 7, 1946

The experimental work along these particular lines was discontinued at this point, since it was clear that a yield more than twice that of the previous synthesis had been obtained, and there remained possibilities for improvement. The yield for the four steps was taken to be 1.0, 0.9, 0.8, and 0.3, respectively, for an over-all yield of 0.21 as compared with the original 0.08.

B. Counting Equipment

As it became increasingly clear that trouble-free operation of the counting equipment would result only from a major overhaul, the work was begun on a piecemeal basis and continued throughout the time while the last of the samples were being counted,

about a year and a half after the injections. The extra number of amplifiers and scaling units made it possible to continue the counting during this work, except for a period of 2 months when the power supplies were reworked.

Since the major annoyance seemed always to be with the scaling units, it was important to provide some means of checking these units independently of the actual counting. It was the general experience that two scaling circuits which were operated in parallel seldom gave the same counts. To settle this question of which, if either, was correct, a pulse generator was constructed. It deserves some comment, since it was a very valuable tool in trouble shooting and in the specific effort of establishing the limitations of the scaling units; it also permitted their eventual reconstruction so that two or more units gave the same number of counts. It had several features not usually found in such units.

The pulse generator provided an output of paired square-wave pulses at a pair frequency ranging from 10 to 30,000 cycles/sec., with the time separation of the two pulses adjustable from 0 to twice the frequency; in addition both pulses were independently variable in sign, amplitude, and duration. The essential details are seen in Fig. 12, as follows: The basic frequency of the pair is controlled by the oscillation frequency of the first 885 tube, this frequency being variable with the aid of the series of condensers 5 and the variable resistor 4. The remaining two 885 tubes become conducting at some point along the saw-tooth wave output of the basic oscillator, the point being controlled by the cathode-bias variable resistors

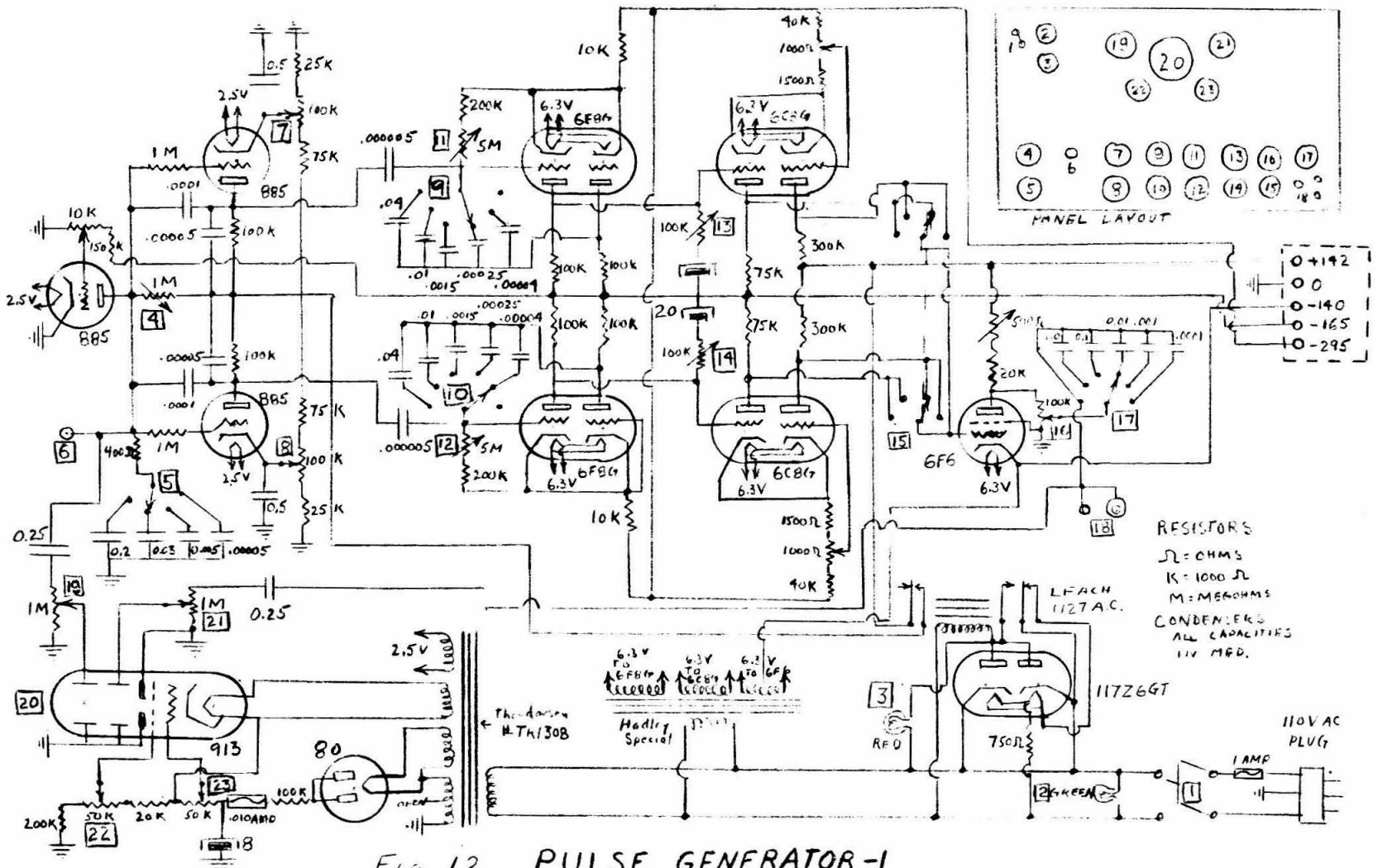


Fig. 12 PULSE GENERATOR-1

7 and 8. The output of these two tubes, if viewed simultaneously, represents a pair of pulses of the basic frequency of the first 885 oscillator, which are separable as desired. The two pulses are then independently shaped, amplified, phased by two separate direct-coupled circuits, each using a 6F8G and 6C8G tube, and finally combined in a common 6F6 output tube. The 6F8G tubes are in simple Eccles-Jordan trigger circuits which provide for the square waves with the control of the duration through the condenser banks 9 and 10 and the variable resistors 11 and 12. The 6C8G tubes are direct-coupled amplifiers and inverters and provide pulses of opposite sign from the two plates, with amplitude control of the pulses through the variable resistors 13 and 14. The double-bank switch 15 provides for any combination of sign of the two pulses (+-, ++, -+, or --) to the direct-coupled output tube, the 6F6, and attenuation of the final output 18 is provided by the resistor 16, the bank of condensers and switch 17 permitting either direct-coupled or condenser-coupled output. The direct-coupled, zero-signal output level is at ground potential, the pulses being either above or below this level as desired. The 913 oscilloscope tube was provided for a simple check of the pulse separation, with the appropriate controls, and the 117Z6GT is interlocked with a 110-volt ac relay in such a manner as to provide a time delay of some 45-60 seconds after the filament power is on before the plate voltage is applied to the 885 tubes.

The power supply for this unit (Fig. 12) is relatively straightforward. The direct coupling required a high degree of stability and was obtained only with the battery-standard regulation

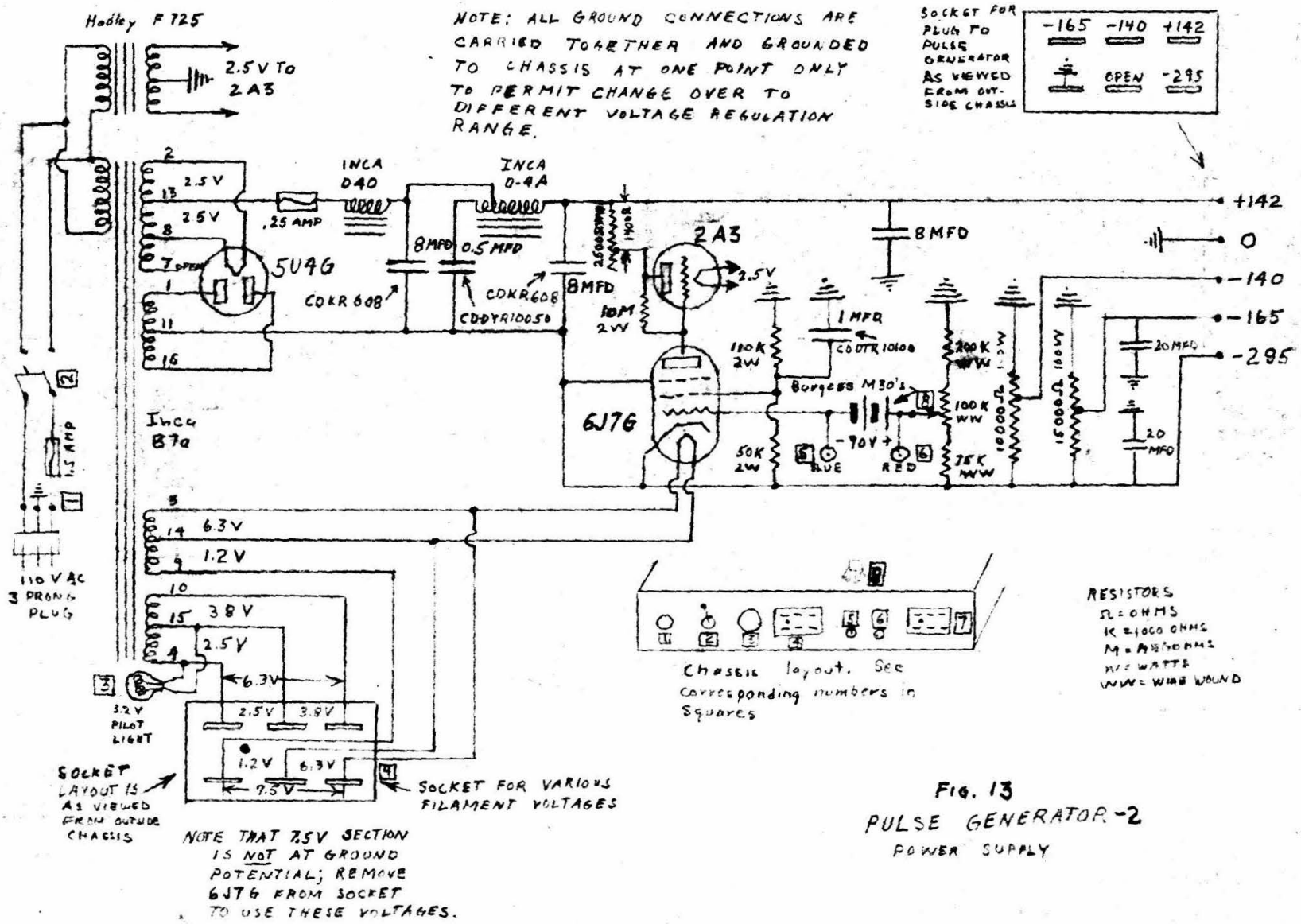


FIG. 13
PULSE GENERATOR-2
POWER SUPPLY

circuit illustrated.

Figure 14 indicates a few of the possible output wave shapes as observed with an oscillograph; it should be remembered that all of these can be reversed in sign and amplitude over the range of pair frequency. It was extremely simple to check the

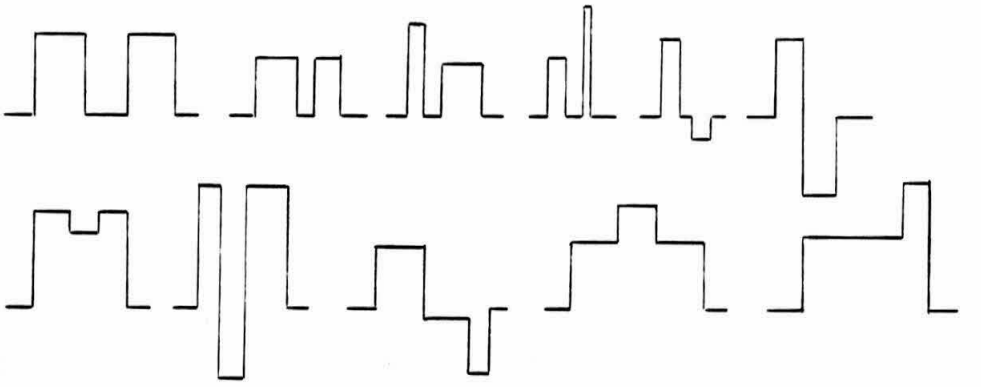


Fig. 14

scaling units by applying a typical paired pulse similar to the first few samples in Fig. 14 and then to decrease the pair separation until the recorder counts decreased because of inability to resolve the pair. The data obtained in this way made it possible to revise the amplifier output circuits to provide for the best possible resolution.

The final counting equipment is completely illustrated in Figs. 15 through 21 and is briefly described to indicate some of the more important features. All of the equipment was on a wheeled wooden table about 24 by 40 inches, with a shelf beneath. Figure 15 indicates the nature of the conduit and boxes built into this table to provide safe and convenient interconnection of the various units with each other and with the power supplies below on the shelf. Figure 16 indicates the main power wiring, together with

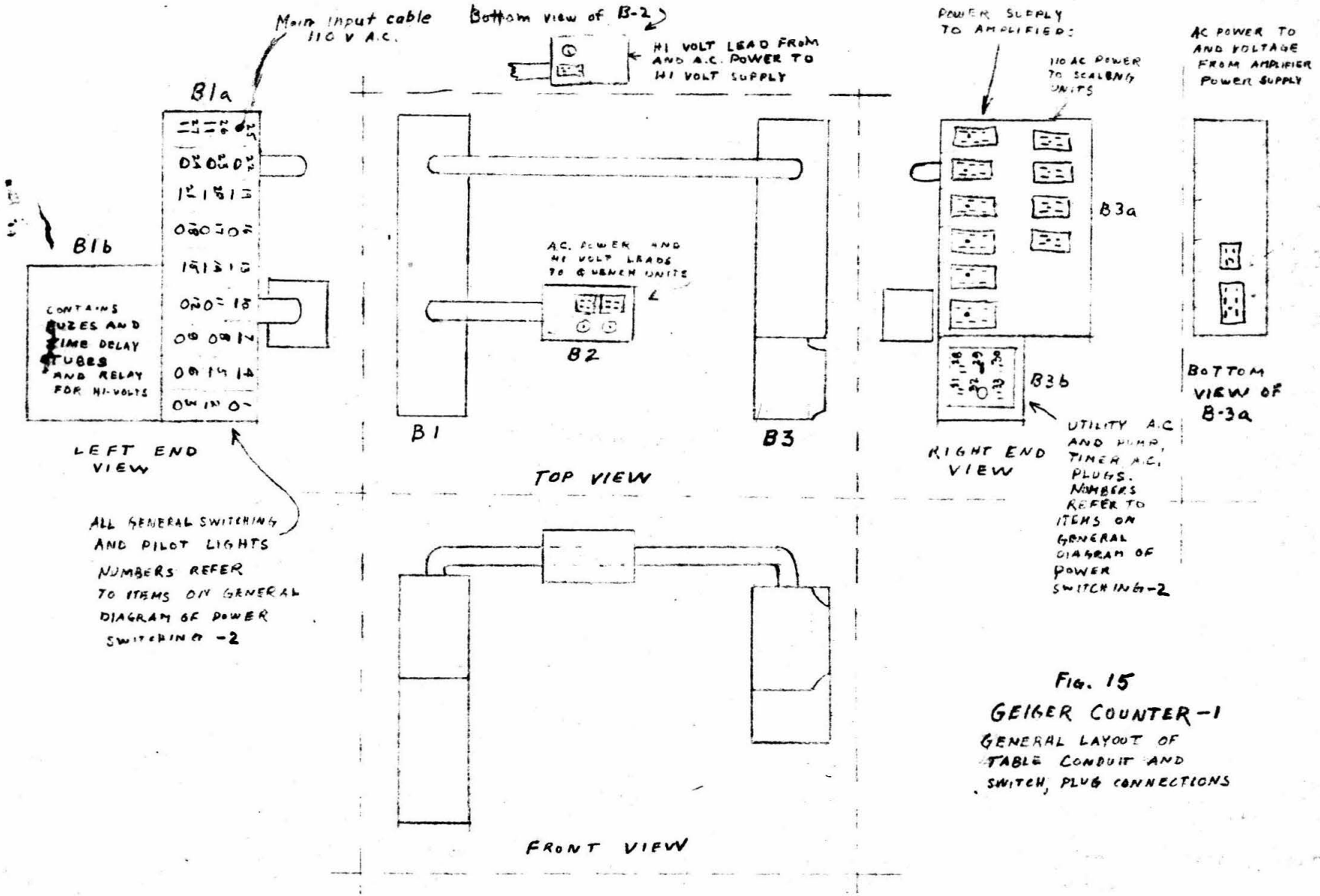


FIG. 15

GEIGER COUNTER-1

GENERAL LAYOUT OF
TABLE CONDUIT AND
SWITCH, PLUG CONNECTIONS

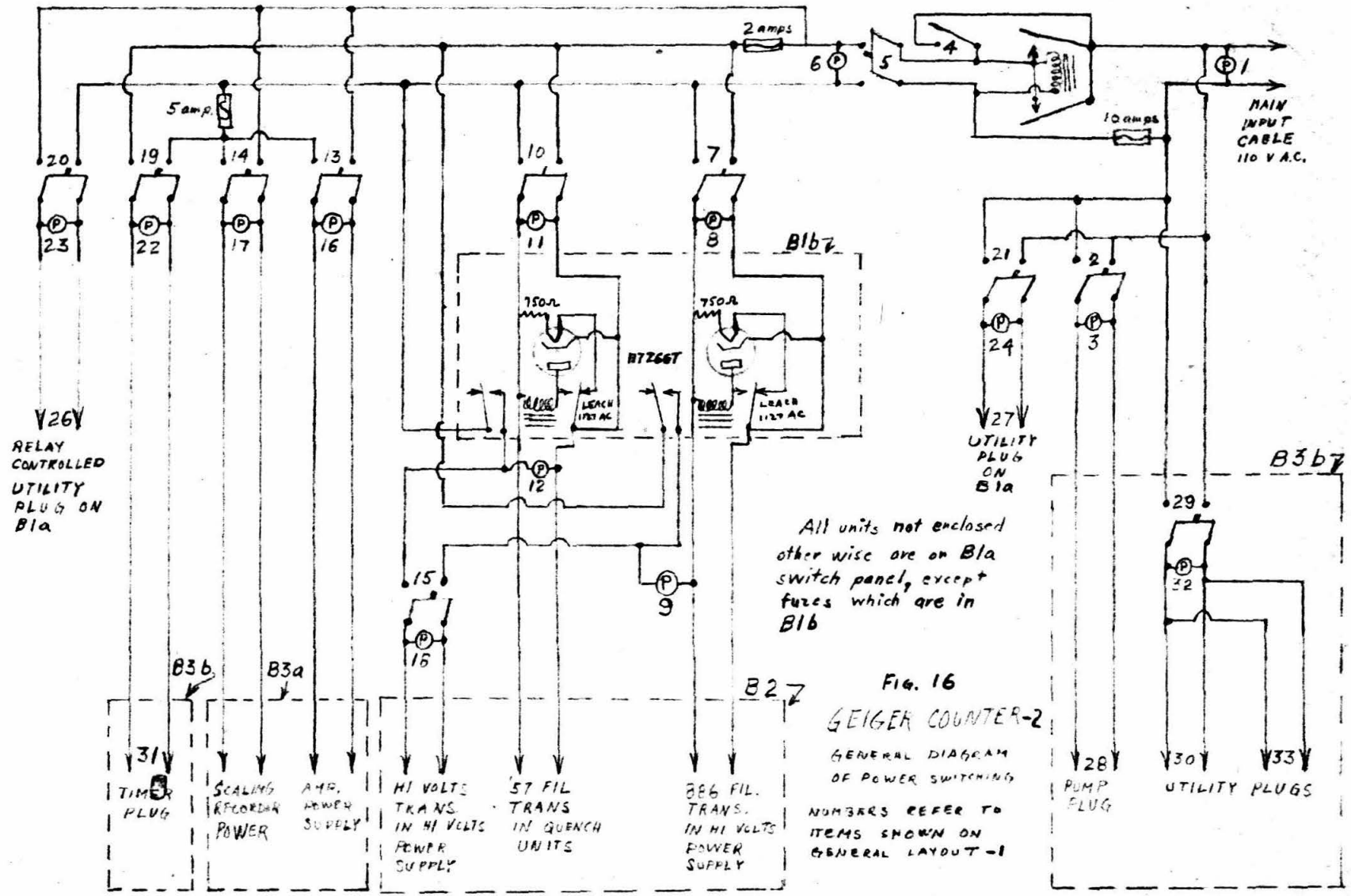


Fig. 16

GEIGER COUNTER-2

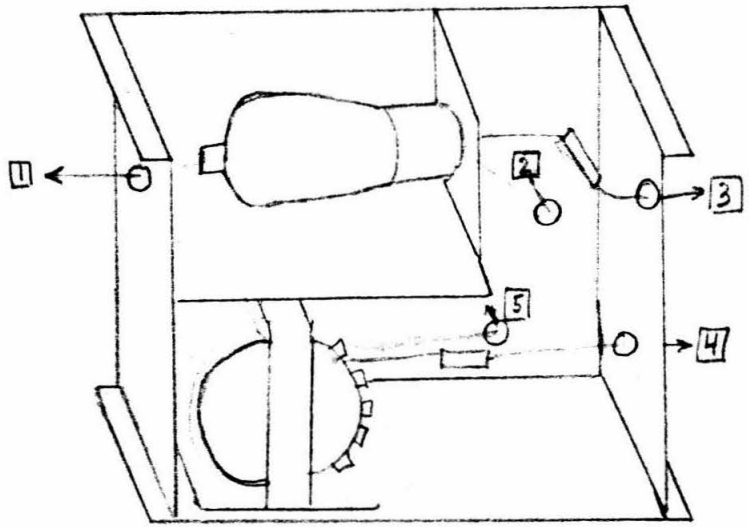
GENERAL DIAGRAM OF POWER SWITCHING

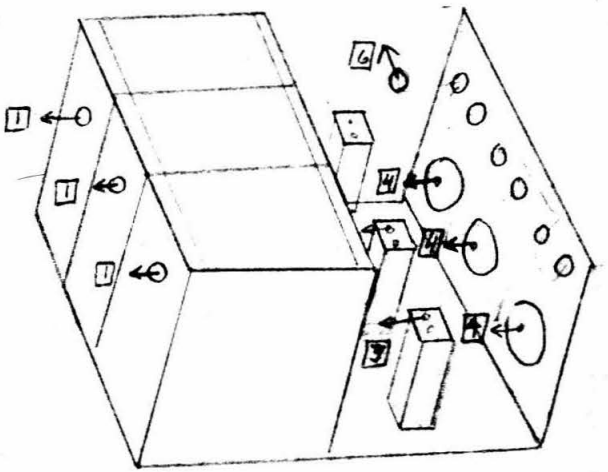
NUMBERS REFER TO ITEMS SHOWN ON GENERAL LAYOUT -1

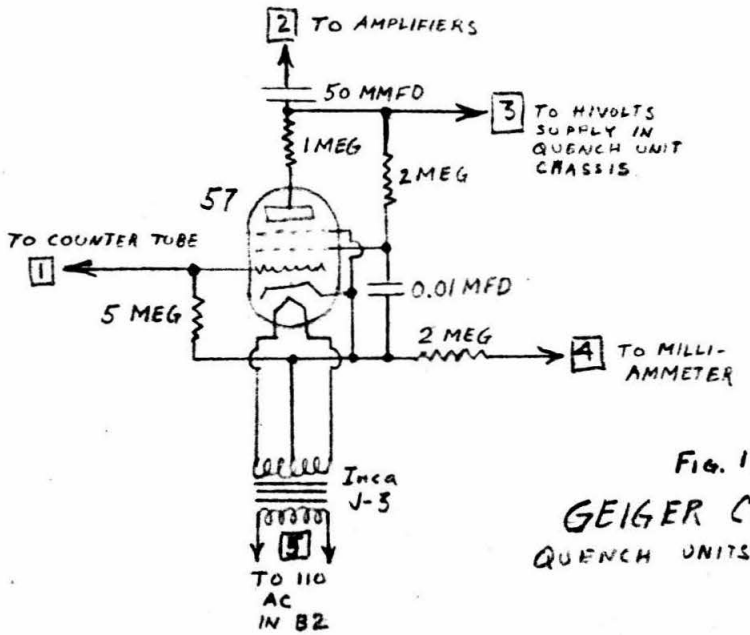
the simple tube and relay circuits that provided the time delay so that high voltage was not applied to the plates of either the 886 rectifiers in the high-voltage supply for the counters or to the 57 tubes in the counter quench circuits until after 45-60 seconds of filament warm-up of both sets of tubes. A lockout relay was provided in the main power so that the equipment would not be restarted in the event of power failure in case it was unattended.

The layout and wiring of the six quench-tube units are shown in Fig. 17. These units were separated from the main amplifiers and put close to the counter bell jar, but otherwise no changes in the original design were made.

The six amplifiers were as shown in Fig. 18 and were rather completely altered. Originally only four tubes were used; after initial amplification and pulse-height clipping of the quench-unit pulse, the output was fed directly to a typical Rossi coincidence circuit with an output plug for connecting into this circuit whatever amplifiers were desired, the remaining tubes providing output to count either the total pulses of the particular quench unit or the coincidence count of two or more units. The revision included the addition of the 6C8G tube which provided two pulses 180° out of phase. The switching permitted either of these pulses to be fed to the coincidence output socket, the remainder of the amplifier responding to either the input counts of the amplifier or the coincidence counts as desired. These pulses of opposite sign permit the







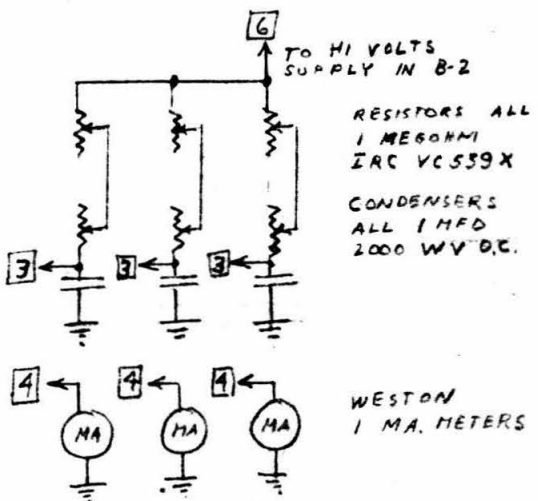
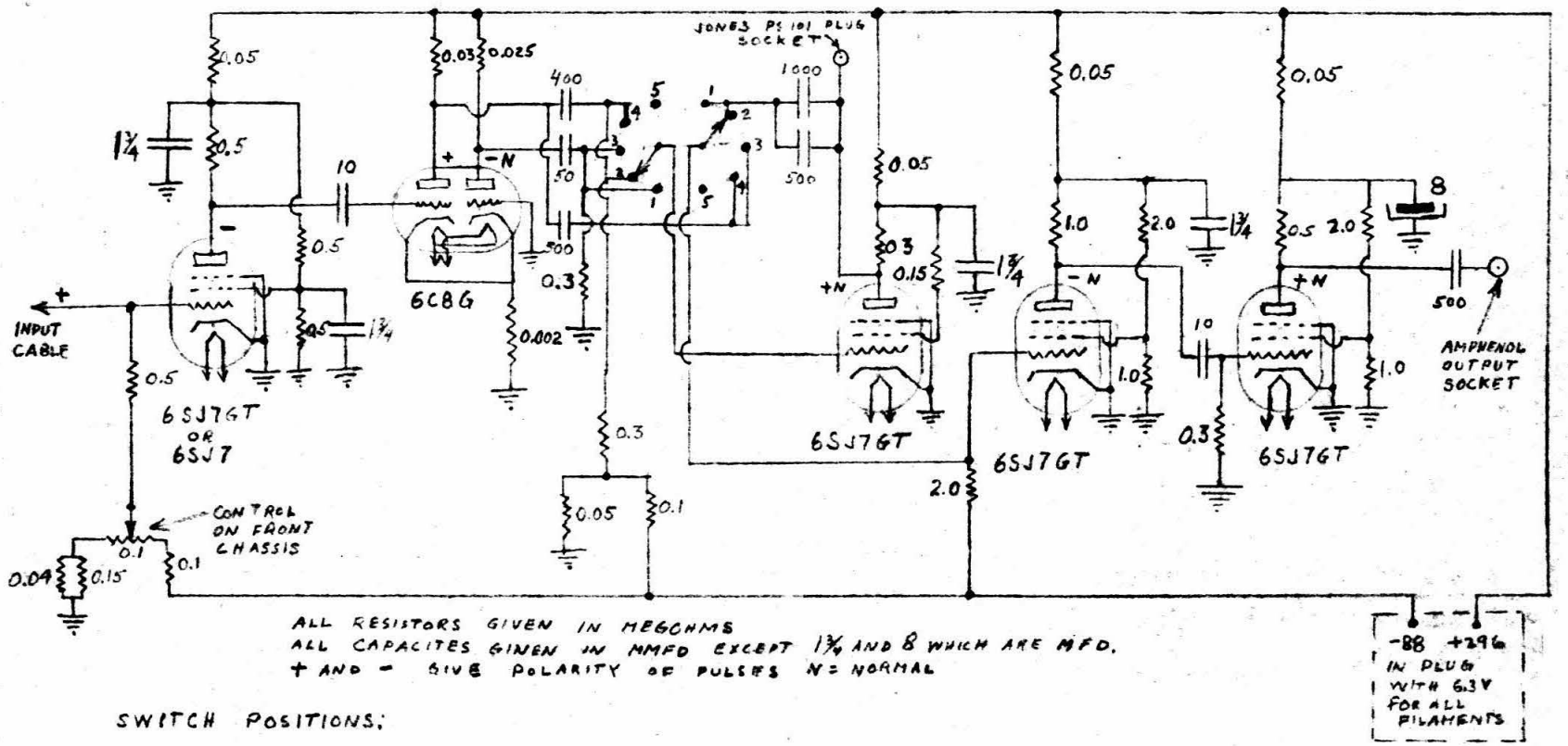


Fig. 17

GEIGER COUNTER-3

QUENCH UNITS AND CHASSIS



ALL RESISTORS GIVEN IN MEGOHMS
ALL CAPACITES GIVEN IN MMFD EXCEPT 1¾ AND 8 WHICH ARE MFD.
+ AND - GIVE POLARITY OF PULSES N = NORMAL

SWITCH POSITIONS:

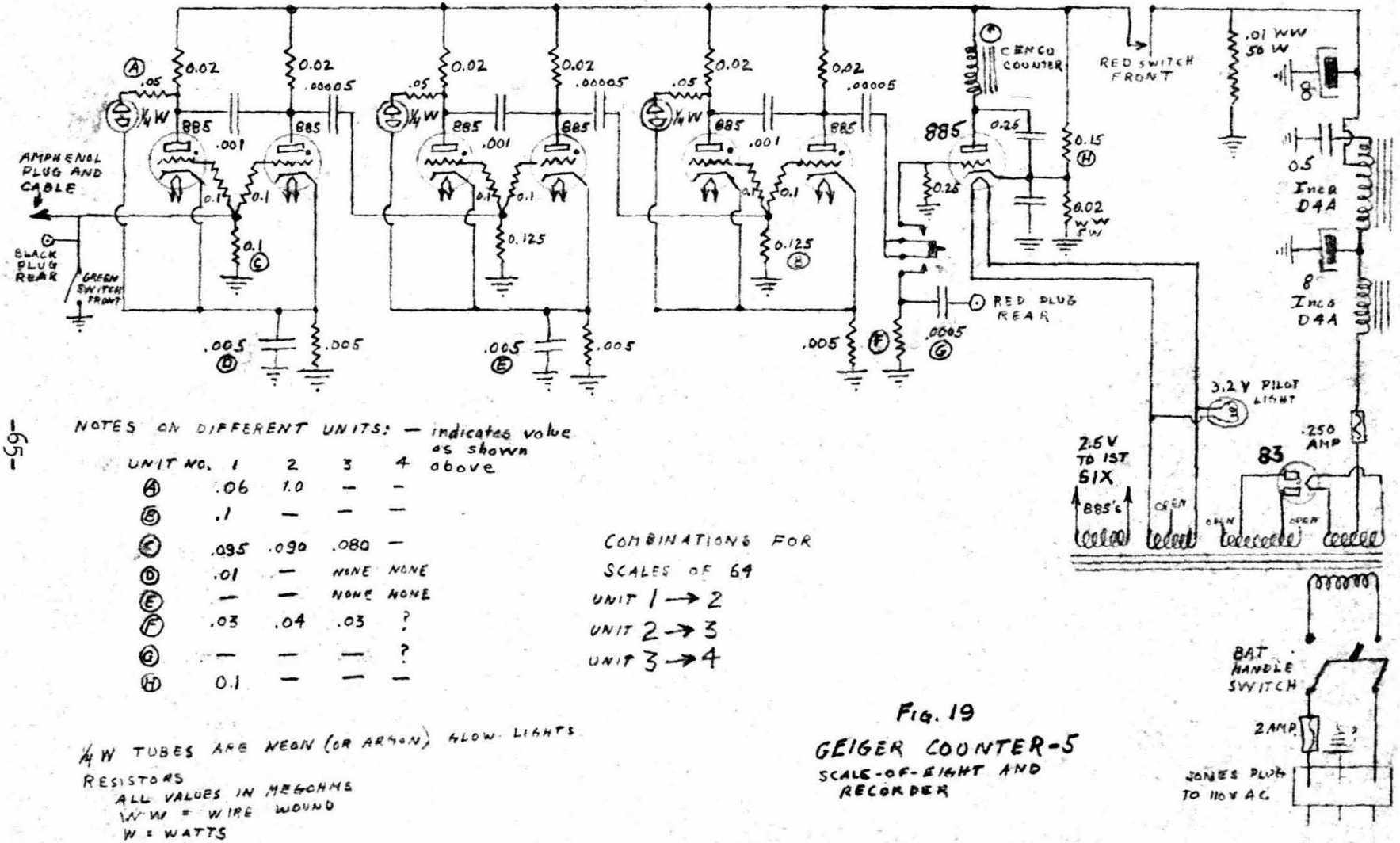
1 Normal Pulse to Rossi circuit; output direct, coincidences if connected to other amplifiers
2 Anti " " " " " " " " " " " " "
3 Normal " " " " " singles this amplifier only
4 Anti " " " " " " " " " "
5 Open.

Fig. 18
GEIGER COUNTER-4
AMPLIFIERS

use of anti-coincidence counting, a brief preliminary study of which was made as described below.

The scaling circuits are indicated in Fig. 19; the main revision here included simple balancing and adjustment of the various circuit components in the individual units to achieve reproducible behavior, as checked with the pulse generator either directly or through the amplifiers. The inherent variation of the thyratron type tubes and their long time constants seem to be an insurmountable difficulty with this type of scaling circuit.

The high-voltage supply for the counters, shown in Fig. 20, was also rebuilt around a battery reference voltage stabilizing circuit. The plateaus of the sulfide-coated counters in the alcohol-air gas were by no means as flat as for the other usual types of Geiger counters, and variations in line voltage introduced considerable variation in the background counting rates with the original supply. Until this supply was constructed, a recording voltmeter was connected to the ac supply, and any long-period counts during which there were serious voltage fluctuations were discarded. The success of the battery reference regulation led to its use in the amplifier power supply (Fig. 21), but it was probably not necessary for any but anti-coincidence work, where the stability of the Rossi circuit parameters is fairly important.



NOTES ON DIFFERENT UNITS: — indicates value as shown above

UNIT NO.	1	2	3	4
(A)	.06	1.0	—	—
(B)	.1	—	—	—
(C)	.095	.090	.080	—
(D)	.01	—	NONE	NONE
(E)	—	—	NONE	NONE
(F)	.03	.04	.03	?
(G)	—	—	—	?
(H)	0.1	—	—	—

COMBINATIONS FOR SCALES OF 64

UNIT 1 → 2

UNIT 2 → 3

UNIT 3 → 4

¼ W TUBES ARE NEON (OR ARGON) GLOW LIGHTS

RESISTORS
ALL VALUES IN MEGOHMS
WW = WIRE WOUND
W = WATTS

CONDENSERS
ALL VALUES IN MFD.

FIG. 19

GEIGER COUNTER-5
SCALE-OF-EIGHT AND RECORDER

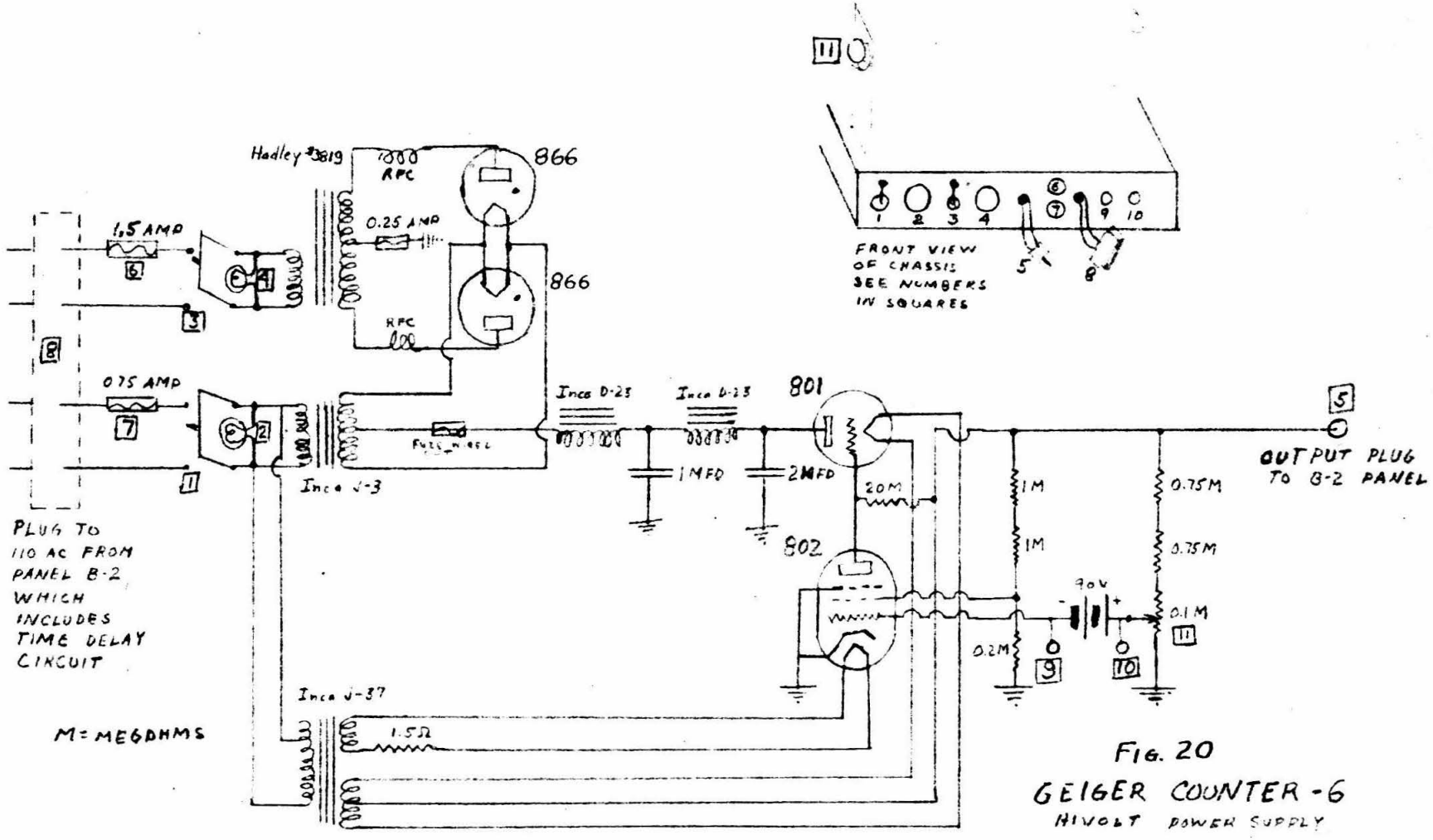
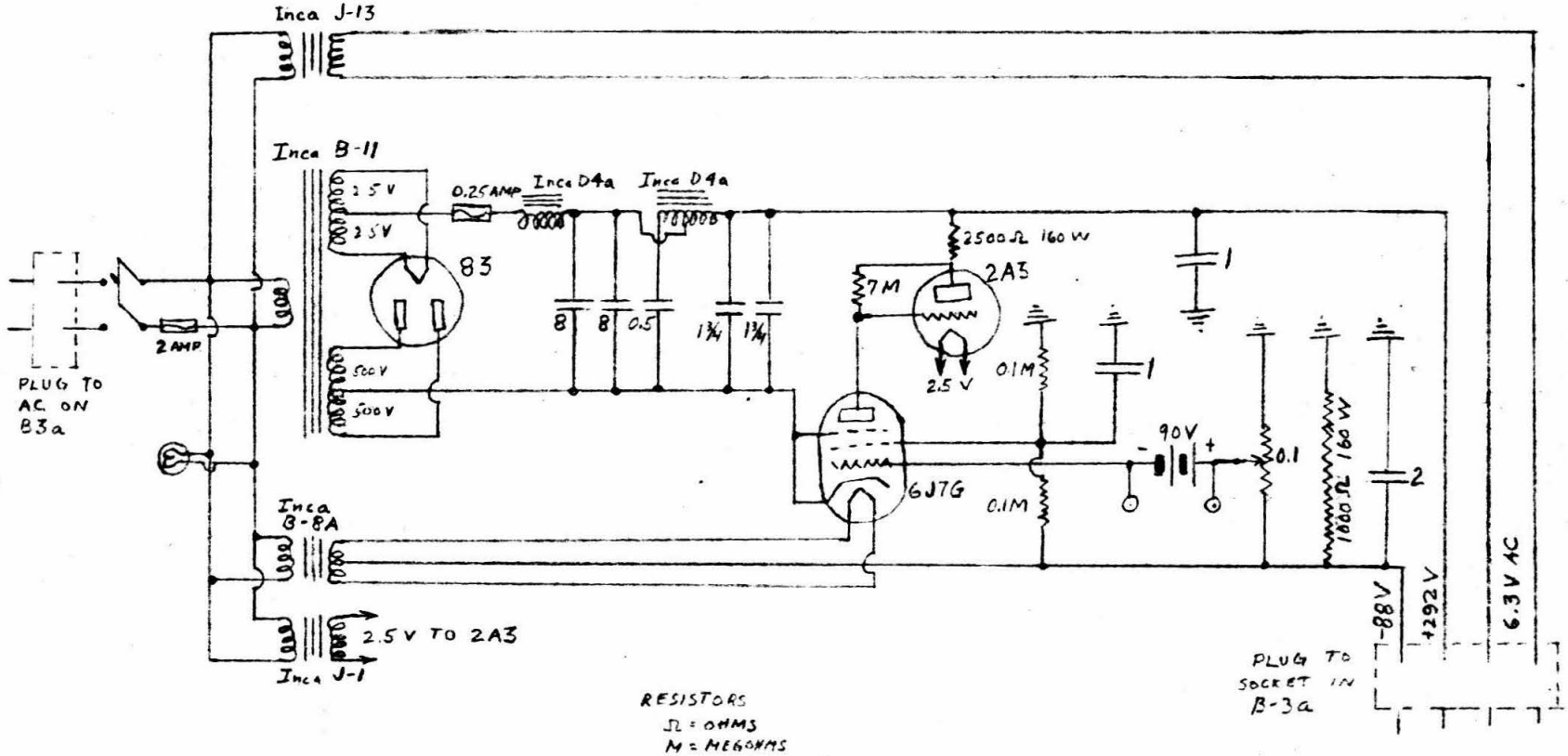


FIG. 20
GEIGER COUNTER-6
HIVOLT POWER SUPPLY



RESISTORS
Ω = OHMS
M = MEGOHMS
W = WATTS

ALL CAPACITIES IN MFD.
8 MFD ARE CC IE B
1¾ " " PAPER
0.5 IS C.D DRY 10050
1 ARE " DA 6100
2 IS " DR 4200

Fig. 21
GEIGER COUNTER -7
AMPLIFIER POWER SUPPLY

C. Anti-Coincidence Counting Experiments

In the counting of very weak samples the use of anti-coincidence methods might prove particularly useful. For this reason the reworking of the amplifiers included the phase-shifting feature in order to permit an experimental examination of the facts. The basic feature of the method is the use of some number of counters which "surround" the single or coincidence sample-counting arrangement; these counters, which the desired sample radiation cannot reach, provide out-of-phase pulses to the coincidence circuit. The result is that the sample-counters register will not respond to any pulse which is simultaneous with one of the anti counters; since most of the radiation which makes up the background of the sample counter is of high energy, it should discharge the anti counters and hence not be registered; depending of course on the efficiency of the counters and the geometry, the background might be substantially reduced.

The general behavior of the circuits themselves was checked with some counters of the cosmic-ray type obtained from Dr. Pickering. Three counters in a vertical arrangement with a weak source of radium directly above gave a triple-coincidence counting rate of 215 ± 2; with the middle counter as an anti-coincidence counter, the counting rate dropped to 5 ± 0.3, indicating the over-all efficiency of the amplifiers and counters to be of the order of 98% for this radiation.

One early experiment indicated that the background rate of the first single counter used in the thiamin work could be

reduced from 1340 to 480 counts per 1000 seconds by six surrounding anti counters; other tests of the method with actual samples were made with slightly different counters.

In one experiment a radiosulfur sample was counted with a single counter surrounded by about six anti counters; the counting rates are shown in Table 12.

Table 12

	Single counter alone	Single counter with anti-coincidences provided from surrounding counters
Background	439 ± 5	181 ± 3.1
Sample S-15	724 ± 5	435 ± 4
S-15 - Background	285 ± 7	254 ± 5
fractional error	.026	.020

As can be seen from the data, the decrease in background of the single counter amounted to about 40%, with only an 11% decrease in the actual number of counts above background from the sample. This decrease is probably to be attributed to the rather long time constants of these counters in the air-alcohol mixture; the anti pulses from so many anti counters produced sufficient accidental coincidences to decrease the counts by 11%. The net increase in precision during a given time amounts to 23%. The analysis of Part II, Section D. 1., can be directly applied to this particular type of system if the subscript c is replaced by the subscript a, for anti-coincidence counts. The factor α is now

defined as c_a/c_s, and the factor R as b_a/b_s, both of which may be obtained from the data of Table 12. $\alpha = 254/285 = .89$; $R = 181/439 = .412$, and hencefor a weak sample, where βs is small in comparison to 1, the ratio of the counting times $T_a/T_s = .52$. This actual system would reduce the counting time 48%.

In another experiment, a combination of coincidence and anti-coincidences was tested; two counters arranged for coincidence counting were surrounded by a bank of six anti counters. For these counters the coincidence background of 21.5±0.5, without the anti counters, was reduced to 5±0.6 with the anti counters; in this arrangement the sample coincidence counts were not reduced. The data are indicated in Table 13; the net increase in precision is 33%.

Table 13

	Coincidence-counters counting rate	
	Without anti	With anti
Background	21.4 ± .6	5 ± .3
Sample S-12	36.7 ± .8	20.1 ± .6
S-12 - Background	15.3 ± 1	15.1 ± .67
Fractional error	.066	.044

It is clear that the anti-coincidence method offers considerable improvement over either singles counting or coincidence counting, but a direct experimental check of the particular system proposed would be required to establish whether a singles + anti system would be preferable to a relatively similar coincidence + anti

system. The utmost that could be expected of the anti-coincidence method would be to reduce the background to very small values, but as was indicated in the previous analysis in Part III Section D, such reduction is not alone a sufficient criterion; as indicated there, the loss in counts from the geometry of the coincidence system may reduce the precision below that obtainable with a single counter.

V. REFERENCES

(1) Jollifee, et al, Am. J. Med. Sci. 198, 198 (39).

(2) Westenbrink and Goudsmit, Arch. Neerl. de Physiol. 22, 317 (37).

(3) Sciclounoff, Compt. rend. Soc. biol. 130, 980 (39).

(4) Najjar and Holt, Bull. Johns Hopkins Hos. 67, (2) 107 (40).

(5) Harris, Leong, and Ungley, Lancet 1, 539 (38).

(6) Ritsert, Klin. Wochschr. 17, 139 (38).

(7) Magyar, Klin. Wochschr. 18, 1157 (39).

(8) Sciclounoff, Compt. rend. Soc. Biol. 131, 53 (39).

(9) Leong, B. J. 31, 367 (37).

(10) Leong, B. J. 31, 373 (37).

(11) Jansen, Rec. trav. chim. 55, 1046 (36).

(12) Hennessey and Cerecedo, J.A.C.S. 61, 179 (39).

(13) Westenbrink, Rec. trav. chim. 56, 803 (37).

(14) Peters and Van Slyke, "Quantitative Clinical Chemistry," Williams and Wilkins, Baltimore, 1932, v.2, p. 892.

(15) Folin, J. Biol. Chem. 1, 131 (05).

(16) Benedict, J. Biol. Chem. 6, 363 (09).

(17) Cooley, Yost, and McMillan, J.A.C.S. 61, 2970 (39).

(18) Rasetti, "Elements of Nuclear Physics," Prentice-Hall, New York, 1936, Chapter 2.

(19) Swift, "A System of Chemical Analysis," Prentice-Hall, New York, 1939, p. 550.

(20) Borsook, Keighley, Yost, and McMillan, Science 86, 525 (37).

(21) Personal communication, Dr. M.L.Crossley, American Cyanamid Co.

(22) Robinson, ed., "Organic Syntheses," John Wiley and Sons Inc., New York, 1939, v. 19, p. 10.

(23) Personal communication, Dr. Randolph T. Major, Merck and Co.