

**Staff Meeting Bulletin**  
**Hospitals of the » » »**  
**University of Minnesota**

**Laboratory**  
**Diagnosis of**  
**Gonorrhea**



I. LAST WEEK

Date: December 16, 1938  
Place: Recreation Room,  
Powell Hall  
Time: 12:15 to 1:20 p.m.  
Program: Movie: "Mickey's Parrot"  
A Walt Disney Picture

## Announcements

The Roentgen Diagnosis of  
Acute Pulmonary Disease

Herman H. Jensen  
Curtis B. Nessa

Annual Report  
Leo G. Rigler

Discussion  
Walter Ude  
Wesley Spink  
Leo G. Rigler

Present: 131

Vacation: December 23, 1938  
December 30, 1938

Gertrude Gunn  
Record Librarian

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II. MOVIE

Title: "That Mothers Might Live"

Released by: M-G-M Miniature

\* \* \*

III. ANNOUNCEMENTS1. WEDDINGS

Stanley Lindley and Jean Steele,  
December 22, 1938.

Carl Lind and Mae Goodell,  
December 31, 1938.

Robert Leighton and Grace  
Zschesche, December 31, 1938.

Congratulations and Best Wishes!

2. BABIES

Eleanor Beatrice Carlson,  
daughter of Dr. and Mrs. Herbert  
A. Carlson, born Tuesday, December  
27, 1938.

More Congratulations!

3. COURSES -

Center for Continuation Study

Ophthalmology, January 16 - 21

Hospital Administration,  
January 23 - 28

Medical Record Librarians,  
January 30 - February 1, 1939

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IV. LABORATORY DIAGNOSIS  
OF GONORRHEA

Milan Novak

Introduction

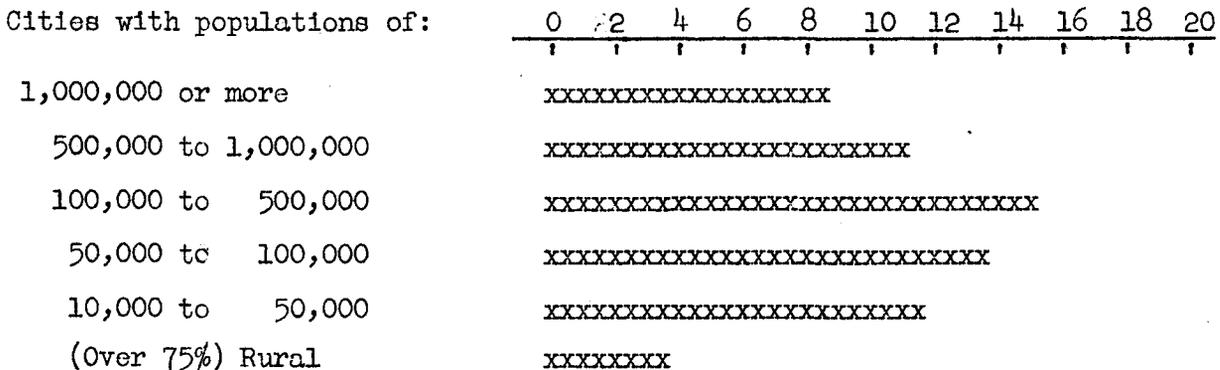
During the past few months, the University of Minnesota Hospitals Bacteriology Laboratory, in conjunction with the Department of Bacteriology and Immunology of the Medical School and Albert Leibovitz, a graduate student, has attempted to improve upon the methods used in the diagnosis of gonorrhoea at this hospital. The object of this presentation is to introduce the method now in use and to stimulate interest and cooperation so that the method can function to the most efficient degree.

Too often the hospital personnel have only a general idea as to how a specimen for bacteriological examination should be collected and submitted. This may be mainly the fault of the laboratory itself since a part of our work is necessarily a matter of education. Frequently the interim between the study of bacteriology during the first year of the medical course and the internship year seems to leave a gap into which most of the bacteriological knowledge of an average medical student disappears. We shall attempt then to go back and pick up some of these fragments and relate the procedures necessary for a thorough examination of material from a suspected case of gonorrhoea according to the most recent standard methods.

Incidence of Gonorrhoea

It is difficult to discover the exact prevalence of an infectious disease among the human race. Gonorrhoea, with its associated social taboos, is a disease in which accurate morbidity rates are especially difficult to ascertain. Certain statements in the literature, however, serve to give some indications as to the widespread distribution of the disease. Pelonze states that estimates indicate 60% to 90% of all males in large cities contract gonorrhoea at some time during life; that 20% of all married men contract the disease during their married life and about one-half of these infect their wives; that 40% to 60% of all operations on the uterus and adnexa are necessitated by previous gonorrhoeal infection. Usilton in 1935 estimated that in the United States there are almost a half million individuals constantly under treatment for gonorrhoea and that there are one million new cases contracted each year. The following chart illustrates the frequency of the infections in various groups.

GONORRHEA RATE PER 100,000 POPULATION





gonococci in cultural media suggests the possibility that they may also assume these characteristics in the human body. One such strain, originally Gram negative but subsequently Gram positive, was recently isolated in our laboratory. After repeated subculture, the organism finally reverted to its original Gram negative character. Several reports of this nature may be found in the literature. While such reports may be criticized on the basis of technique, the number of reports in the literature together with our own experience along this line convinces us of the unreliability of a diagnosis made on the appearance of the stained smear alone. Hence, the common practice of reporting what is seen on the slide rather than making a definite diagnosis is warranted and must be interpreted by the physician in the light of the history and clinical findings. Culture, on the other hand, if positive, affords a definite diagnosis.

A number of workers have pointed out the advantages afforded by a correctly made smear. The swab containing the diagnostic material should not be stroked on the slide but should be rolled gently in order not to disrupt the leucocytes. Too great an emphasis cannot be exerted on this one point, since it facilitates greatly the observation of the characteristic diplococci within the leucocytes. The phrase "a g.c. smear" should therefore not be taken literally, since such a preparation presents fragmented leucocytes in concentrated or rarified numbers on the slide. Gram stains of such preparations show a wide range of reaction to the staining process since thin areas are decolorized too much while thick areas may still retain the violet dye. The examiner is then forced to take his choice of either the Gram positive or Gram negative organisms on the slide. Stained slides from acute cases are usually easy to read microscopically. However, chronic cases present a much more difficult problem since a more diversified flora exists, especially in slides from females. It is in these cases that the bacteriologist is most likely to err, if only a slide preparation is examined.

In this laboratory, slides submitted for examination are first fixed by gentle flaming in the usual manner. They are then stained by Burke's modification of the Gram staining procedure, which gives a more definite picture than that obtainable by the ordinary Gram stain. Slides are examined, and a report is made as to pus cells present, the morphological characteristics of the bacteria present together with their relative numbers, and their relationship to the leucocytes as to position within or without the cells. The interpretation is reserved for the physician.

## 2. Cultural methods

Although the successful cultivation of gonococci in pure culture dates back to 1885 (Bumm), the development of the cultural method as a diagnostic aid occurred but a few years ago. Leahy and Carpenter state that, in a survey conducted in 1936, only 23 out of 145 public laboratories reported successful isolation of the organisms. A considerable impetus to the cultivation of gonococci was made in 1934 when McLeod and his coworkers showed that the cultural method is superior to the smear method, especially in chronic cases in the male and in both acute and chronic cases in the female. At the present time, many similar reports exist in the literature, so that the advantages of the cultural method over the smear method alone are beginning to be more generally recognized. The following reports are worthy of perusal.

RESULTS OBTAINED BY SMEAR AND CULTURE METHODS IN  
A GROUP OF TREATED AND UNTREATED FEMALES

McLeod et al., J. Path. & Bact., 1934.

<u>Year</u>	<u>No. of Cases</u>	<u>Positive by culture</u>	<u>Positive by smear</u>	<u>Positive by both culture and smear</u>
1931	687	10.77%	5.09%	12.08%
1932	540	16.50%	10.00%	20.00%
1933	579	15.20%	9.50%	20.00%
1934	256	19.90%	6.30%	21.90%
Total	2062	14.50%	7.75%	17.60%

USING SPECIMENS FROM BOTH THE CERVIX AND URETHRA:

Both positive in smears: 7%  
Both positive in cultures: 22%

MOST STRIKING RESULTS IN CERVICAL EXUDATES:

Smears positive: 16%  
Cultures positive: 33%

DIAGNOSIS OF RECTAL GONORRHEA IN WOMEN WITH GENITAL GONORRHEA

Ruys, J.A.M.A., May 1935

Of 82 women with genital gonorrhoea, 48 also had rectal gonorrhoea, and of 38 children with vulvovaginitis, all had rectal gonorrhoea as disclosed by cultural methods:

Smear positive, culture positive	48	
Smear positive, culture negative	3	
Smear doubtful, culture positive	23)	
Smear doubtful, culture negative	3)	72
Smear negative, culture positive	46)	

In 72 cases where smears were negative or doubtful, cultures were positive in 69.

Conclusion: cultural method very superior to smear method in areas where there is heavy contamination with other organisms.

## A COMPARISON OF CULTURE AND SMEAR METHODS IN THE DIAGNOSIS OF GONORRHEA

Leahy and Carpenter, Am.J.Syphilis, July, 1936.

Method of culture:	Chocolate agar in 10% CO <sub>2</sub>	
Total number of patients:	138	- 90 females, 48 males
Specimens total	237	
Smears positive	106	- 45%
Cultures positive	130	- 55%
Cultures positive with smears negative or doubtful	30	- 13%
Cultures negative with smears positive	5	- 2% (cultures taken soon after fever therapy)
Cultures superior to smear	24	- 10%

## COMPARISON OF CULTURE AND SMEAR DIAGNOSIS OF FEMALES

Carpenter, et al., Am. J. Syphilis, Jan. 1938.

	<u>Number positive</u>	<u>Percentage positive</u>	<u>Percentage of total positive</u>
Total number positive findings using both smear and culture methods	223	100%	
Culture positive, smear positive	89	39%	
Culture positive, smear negative	116	52%	
Culture negative, smear positive	18	8%	
Total number positive cultures	205	100%	92%
Cultures positive, smear positive	89	43%	
Cultures positive, smear negative	116	56%	
Total number positive smears	107	100%	48%
Cultures positive, smear positive	89	83%	
Cultures negative, smear positive	18	16%	

## SUMMARY

Superiority of cultural over smear method	191%
Superiority of both methods combined over the smear method alone	208%

### Choice of media

Perhaps the greatest cause for the delay in the adoption of the cultural method as a diagnostic aid has been the advocacy of diversified and complex media on which to grow the gonococci. In reviewing the literature, one finds such preparations as egg albumin medium, horse liver and white beef extract, powdered bone and egg medium, veal medium, incubated fertile hen's eggs, whole human blood agar, chopped testicle and rice water, horse heart medium, nutrient agar plus juice of green cabbage leaves, aqueous extract of horse penis reinforced with lemon juice and grape sugar, heated blood agar or what is more commonly known as "chocolate" agar. The difficulty in reproducing many of these media is fairly obvious. Out of this somewhat diverse menu, McLeod chose chocolate agar because of the ease with which it can be prepared, and duplicated the good growth obtainable on such a preparation. The medium was first introduced by Cohen and Fitzgorald in 1910 for the cultivation of Hemophilus influenzae, and they termed it "chocolate" agar because of its color. It was first used for cultivating gonococci by Ruediger in 1919 who observed that the gonococcus failed to grow as well on medium containing unheated blood as on that to which heated blood had been added. This observation was repeatedly made by other investigators, and we have noted the same phenomenon. While most strains will grow on unheated blood agar, the colonies are so small that a magnifying glass is often necessary to see them, whereas this is never the case when "chocolate" agar is used. Likewise autoclaved peptones have been shown to have an inhibitory effect on the growth of the gonococcus due to the presence of some oxidized substance produced by heat sterilization of the medium. This substance is removed in the presence of heated blood, but remains in the medium to which whole blood is added. "Chocolate" agar is therefore universally recommended. It is prepared by adding 1 cc. of whole blood to 10 cc. of melted agar at 45-50°C., heating the mixture until it turns a chocolate brown color, and then pouring the contents into a Petri plate. The medium

should be freshly prepared to insure a moist surface on which to streak the material to be cultured. This seems to be essential for success.

A product produced by the Difco Laboratories in cooperation with Carpenter, McLeod and Herrold, is available on the market. It consists of an agar base containing a special proteose peptone, salt, glucose, and a buffer. This makes a convenient medium, easily prepared, to which it is necessary to add only heated blood. We have found this product superior to any other medium for the primary isolation of gonococci from pathological sources.

### Carbon dioxide tension

A great many investigators have noted the stimulating effect of an increased carbon dioxide tension in the gaseous environment on the growth of gonococci. Similar reports exist as to the preference for a decreased amount of oxygen in atmosphere, although there are some reports to the contrary. McLeod and his staff concluded from numerous trials that old laboratory strains of gonococci do not require any alteration in the gaseous environment but state that 8% carbon dioxide is definitely beneficial where primary isolations are being made. Leahy and Carpenter state that 15% of their strains failed to grow without 10% CO<sub>2</sub>. They therefore recommend its use.

Several methods are used for producing the desired amount of carbon dioxide. The cultures can be grown in a Petri plate simultaneously with a spore forming aerobe, or a handful of dampened oats may be placed in the same container, or the inoculated plates can be put into a closed receptacle with a lighted candle which generates carbon dioxide and decreases the oxygen. Since these methods afford a rather uncertain concentration of desirable gases, the method of preference is to add a definite volume of carbon dioxide directly from a cylinder of the compressed gas to a closed receptacle into which the cultures

have been placed, or on the other hand to generate the required volume of the gas from a measured amount of sodium bicarbonate and sulphuric acid placed in a dish inside the receptacle containing the inoculated plates. The latter method is used in this laboratory because of its simplicity and inexpensiveness.

#### Collection of material for culture

Since gonococci are very sensitive to desiccation, exudates and swabs should be cultured immediately. Where the material has to be transported a short distance on swabs, it is highly essential that the swabs be placed into tubes containing about 2 cc. of sterile ascitic fluid or a special peptone solution (Proteose peptone No. 3 - Difco Co.). Such specimens should be inoculated on media as soon as possible, preferably within two hours. The surface of two "chocolate" agar plates is simply streaked with the swab or with material from the fluid in the tube. The outstanding drawback to culture as a method of diagnosis is that, at present, it necessitates proximity to the laboratory, whereas smears can be easily sent any distance through the mail. The development of a preferential medium for the gonococcus such as Loeffler's for the diphtheria bacillus would be a definite advantage to physicians remotely situated from suitable laboratories.

#### Incubation

The common use of a 37°C. incubator for the cultivation of pathogens is based on the belief that these organisms require the same environmental temperature as that of the body. It is well known, however, that external surfaces of the body are several degrees lower than this, and therefore Carpenter has shown that some strains of gonococci grow better at a temperature 2° to 3° lower than that of the body. The recommended procedure is to incubate one plate at 37°C. and another at 34°. In our experience we have failed to note any difference in colony growth at the two temperatures.

Cultures are incubated 36 to 48 hours.

#### Examination of cultures

To an experienced observer, colonies of gonococci are readily recognized. They are grayish, opalescent and slightly convex, and closely simulate the "dew drop" colonies of the meningococcus. The colonies may have fine radial striations and the consistence is sticky and mucoid. They are from 1 to 3 millimeters in diameter and have undulating margins. They must be differentiated most often from diphtheroids, less frequently from streptococci.

Suspicious colonies are stained by Gram's method, and if typical Gram negative diplococci are found, the colonies are inoculated respectively on 1% glucose, maltose, sucrose, and lactose ascitic fluid agar containing Andrade's indicator for confirmation on the basis of carbohydrate fermentation. Gonococci typically ferment only glucose and are thus differentiated from other members of the Neisseria group.

#### The Oxidase test

When the presence of gonococcal colonies is not obvious on direct inspection, the oxidase reaction is resorted to. This test was first described by Gordon and McLeod, also Ellingworth, McLeod and Gordon, in 1928, and has been an invaluable aid to diagnosis in heavily contaminated plates where only a few gonococcus colonies develop. Such plates are usually encountered in cultures from chronic cases where secondary infection has developed.

The test consists of flooding the plate with a 1% aqueous solution of dimethyl-paraphenylene diamine hydrochloride and quickly pouring off the excess. In about 1 to 2 minutes colonies of gonococci turn pink, then maroon, and finally black. Subcultures should be made from colonies in the pink stage as they are usually dead as soon as the darker color develops. Gram stains of oxidase positive colonies are also made.

Other organisms giving the oxidate reaction are some spore-formers, pyocyaneus, and other members of the Neisseria group. The latter group must be differentiated further on the basis of sugar fermentations, since occasionally these organisms can be present on the genitalia. The other organisms are rods and therefore not easily confused. Likewise colonies are much larger. The advantage of the oxidase test over direct inspection alone is shown in the following statistics by Leahy and Carpenter.

Total cultures examined	146
Inspection positive ) oxidase positive )	125 or 86%
Inspection negative ) oxidase positive )	21 or 14%

The test is especially valuable in heavily contaminated cultures such as are frequently encountered from chronic cases in females. Often in such circumstances the gonococcus colonies are completely covered by staphylococci and are therefore impossible to detect by inspection. After application of the dye, these colonies appear as small darkened areas within a large colony of staphylococci. Subcultures and Gram stains from the area are made to confirm the diagnosis.

Confirmation of the presence of gonococci is carried out as mentioned above on the basis of carbohydrate fermentation and also on the failure of the gonococcus to grow on plain agar while nonpathogenic Neisseria grow well.

In some clinics, the typical appearance of gonococcus colonies which give a positive oxidase test and are Gram negative in character constitutes a positive diagnosis. However, considerable experience is necessary for a diagnosis based on this abbreviated routine.

### Reporting Results

The successful isolation of the gonococcus by the above outlined routine

constitutes a positive diagnosis of gonorrhoea and is accepted as indisputable evidence of the disease by courts. In medico-legal cases, therefore, it is obviously necessary to carry the complete routine including the fermentation tests.

Cultures are reported either as "no gonococci isolated" or "Neisseria gonorrhoea isolated in pure culture."

### 3. Serological Methods

#### Precipitin tests

The precipitation test as an aid to diagnosis of gonorrhoea has not been studied extensively. A few reports in the literature indicate that the reaction is not always specific. Several methods of approach have been tried. Monovalent and polyvalent gonococcal autolysates have been used against the serum from chronic cases of gonorrhoea. However, in many cases a non-specific precipitate occurred between the patient's serum and normal saline solution. Also, some sera from patients without gonorrhoea gave a precipitate. Using gonococcus immune rabbit serum, Robinson and Meader demonstrated precipitates with an antigen composed of autolyzed material from secretions obtained from the urethra or vagina. This method gave a fair degree of correspondence with the clinical diagnosis. However, in the diagnosis of chronic cases, it is not possible to obtain enough secretion containing antigenic material to give a positive case, and in acute cases the smear-culture method is more simple to carry out.

#### Flocculation tests

Because of the popularity of flocculation tests for syphilis, a similar method for diagnosing gonorrhoea has been tried. Three general types of antigen have been used: (1) a non-specific antigen using a modified type of beef heart extract similar to that used in the flocculation test for syphilis, (2) a specific antigen made

of a suspension of several strains of gonococci, and (3) a combination of the above two antigens. The tests are carried out much the same way as the tests for syphilis. Varied reports as to the efficiency of the method exist in the literature, but from the many nonspecific reactions that occur together with the failure of positive reactions with serum from known gonococcus cases, a great deal of discredit is cast on the procedure. It has not become popular as have the various flocculation tests for syphilis.

### Complement-fixation

In 1906 a few months after the discovery of the etiological agent of syphilis, Wassermann made a practical application of the already known phenomenon of complement-fixation for the diagnosis of syphilis. In the same year the test was used in an attempt to diagnose gonococcal arthritis after preliminary experiments had shown the presence of complement-fixing antibodies in the serum of rabbits immunized with gonococci. Until very recently considerable apathy has been demonstrated to the procedure in this country, although the test has come to be as routine as the Wassermann in Britain and the continent. This may be explained partly on the basis of technical difficulties encountered in performing the test, since the complement-fixation test is the most delicate of all common laboratory procedures. Since 1931, many papers have been published on results of the procedure, and although the test is not yet perfect it seems to be of some value in certain types of gonococcal infections. A higher percentage of positive results are obtained in infections of long standing, and it is in just these cases that other methods of laboratory diagnosis fail. Cohn (1937) has shown that cases of epididymitis, salpingitis, oophoritis, and arthritis show the strongest reactions and pointed out the fact that smears and cultures from such cases are usually negative. In urethritis and cervicitis, the reaction was weak or negative. The antigens used are usually suspensions of several strains of gonococci, since it has been repeatedly shown that gonococci are a very diverse immuno-

logical group. The hemolytic system used is the same as for the Wassermann, as is likewise the source of complement.

The results obtained seem to indicate that negative reactions are most common in acute cases and that the percentage of positives increases as the infection becomes more chronic. However, the number of negative reactions in chronic cases varies from 10% to 60%, as reported by various laboratories. If we consider the fact that very often a positive complement-fixation test is had on serum from patients who have never had gonorrhoea (from 20% to 70% of normal sera give a positive reaction as reported in various papers), the interpretation of the results is sometimes difficult if not impossible.

The confusing status of the literature is due to the fact that many variations of the test are used and the sensitivity of the reactions differ. This has been demonstrated where samples of a number of sera from known negative and positive cases were sent to several laboratories simultaneously. In most instances, a correlation of about 50% was obtained on the results so determined, which would seem to indicate that the test is of little value at present. Perhaps better results will be obtained when a more standard procedure is developed. A cooperative investigation is now in progress by eight different laboratories in an effort to compare results of the complement-fixation test as carried out on the same specimens of blood from patients so isolated that reinfection cannot occur. This study may lead to a standardization of the test and to improvement of its specificity. The complement-fixation test is not routinely carried out at this University, nor is it done by the State Board of Health.

### Conclusions

1. The cultural method for the diagnosis of gonococcal infection is more reliable than any other laboratory method.
2. The combined examination of direct smear and culture represents the proce-

dure which should give the largest number of positive results.

3. The isolation of *Neisseria gonorrhoea* makes an unquestionable diagnosis and is accepted as irrefutable evidence in court.

4. The smear method when used alone is simple, rapid, and inexpensive, but leads occasionally to false positive and false negative results.

5. Serological methods for diagnosis of gonorrhoea are unsatisfactory at present.

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V. GOSSIP

The holidays are over, and the supply of soda is being replenished. The Christmas season, true to predictions, reached an all-time high in hospitality and good cheer. The Minneapolis Traffic Club's arrangement for the patients was carried off with the usual precision and thoughtfulness. Santa Claus functioned efficiently for the 14th consecutive year. Prior to the trimming of the trees the group assembled for dinner and a program in the hospital dining room. At the nurses' party, a slightly different version of Santa Claus had a shrill voice and an elastic shape. The presentation of gifts was accompanied by unusually appropriate verses. There seemed to be slight hesitation in the response to come forward until the group of girls who were engaged were asked to come up. An amazing number came forward without hesitation to claim the honor and an assortment of rolling pins, and other implements of war. This was the day when yours truly consumed the five meals from noon on. Terrible! The party for the patients was unusually successful, and everyone was remembered. In keeping with the spirit of the occasion, three of our fine young ladies finally consented for better or worse. Grace Zsiesche (former technologist in Main Lab.) was married on the last day of the old year. The same day was selected by Mae Goodell as Jean Steele strutted on December 22nd. The most unusual present was received by William T. Peyton at the Christmas party for the surgical department. An animal with four wooden legs, a pair of shoes on the front and ladies galoshes on the rear, horns attached directly to the spine, and a body of bur-lap, was an appropriate reminder of the mighty hunter's triumphs. From the little bell on the front to the tinsel tail on the rear, there was evidence that care and precision had been exercised in assembling the creature. To intern Hanns Schwyzer goes the credit for the taxidermy. Bill, himself, enjoyed it so much that he invited all of his friends for a piece of venison. Those who came, expecting to have a juicy roast for the holidays, found that the animal had changed to a horse when Bill gave them the well-known horse laugh. Barney Watson, with a fine tree as

a starter, sawed off the top in order to get it in the room with the result that all the boys and girls played "round and round the Mulberry Bush." At the Center party, there was a variation of tacking the tail on the donkey: Ask me for details of this one, which is very good for any occasion. Bob Schenck hardly recognized himself as he came forward to get his gardenia from his good friends, the nurses. "For many favors during the past year." In order to get his name to rhyme, they had to make it sound like something that didn't smell very pleasant. Charlie Rea trimmed his own little tree which was said to be the most unusual tree that had ever been trimmed. Nan Fleming celebrated by turning in her old car and getting a new one. Head Bookkeeper Zula Nesbitt now has an aquarium to catch her wandering eye as she sends out the staff bills for meals consumed with the startling admonition "to pay all hospitals bills in advance." Cecil Watson relieved his days of temporary bachelorhood by going home to the folks to wait for Santa Claus. Moses Barron celebrated Christmas day with his daughter, the same being her birthday, which only goes to prove that the old bird gets mighty mixed up in this business of picking out birthdays. Superintendent Amberg invited his friends to watch the old year out and the New Year in and went to sleep in the process. Leonard Lang could hardly wait until next Christmas rolls around when he really will strut his stuff. Irvine McQuarrie was busy playing the role of a prospective grandfather as his family prepared for the union of the Balfour and McQuarrie clans. Wally Ritchie fell down trying to demonstrate to his family the proper method of coming around a corner on skis with the result that he walks like old man 1938 himself. Ruth Boynton took time off before going to the Health Service meeting in New York to insult Barney Watson with a panel of pictures in which Ruth and Helen Watson appear with weighty strings of fish and Barney is seen with a minnow. The Riggers put on a "family" musical recital in which all the participants were the children of their neighbors....My wish is that the Christmas spirit will remain with us throughout 1939.