KAHN PRECIPITATION TEST WITH SPECIAL REFERENCE TO THE HEATING OF SERUM

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KAHN PRECIPITATION TEST

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THESIS

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WITH SPECIAL REFERENCE TO THE HEATING OF SERUM.*

In the Kahn and Wassermann tests it is customary to heat the serum for 30 minutes at 56°C. before carrying out the test. In the case of the Wassermann test the heating treatment is termed "inactivation" since it is the means of destroying the native complement. In the Kahn reaction, on the other hand, in which complement does not play a role, heating is resorted to because it actually increases the sensitiveness of the serum. Therefore the term inactivation is not properly used in this test. Regarding this increase in sensitiveness Kahn has shown that serum heated at 56°C. is more sensitive in his test than unheated serum. No adequate explanation for this increase in sensitiveness has yet been proposed.

In the present paper, Kahn's studies on the phenomena of precipitation are discussed, the essentials of Kahn procedures with serum are reviewed, a summary of previous experiments on the effect of heating serum is given, and some new experiments on the heating of serum are presented. These last mentioned experiments represent an attempt to determine whether the results obtained by heating the serum above 56°C. for a relatively short period of time might not parallel the results obtained in the regular procedure, in which the serum is heated at 56°C.

*In presenting the theoretical and technical aspects of the Kahn test, reference is constantly made to the two books listed as (1) and (2) in the bibliography.

for 30 minutes. Any reduction in the duration of the heating period would be of practical importance, especially in blood transfusions or emergency surgical cases.

KAHN'S STUDIES ON THE PHENOMENA OF PRECIPITATION.

In the Kahn test for syphilis, serum is mixed with antigen suspension in one or more proportions. The mixture is shaken under standard conditions, physiological salt solution added, and the results read on the basis of the presence (positive serum) or absence (negative serum) of a precipitate. The antigen used is a specially prepared alcoholic extract of dried heart muscle which is mixed with physiological salt solution to form the antigen-saline suspension.

Practically all alcoholic extracts of animal tissue, when properly mixed with syphilitic serum, will produce precipitates after a certain amount of incubation. Therefore it would appear that to evolve a precipitation test for syphilis would be an easy procedure. However no precipitation test had met with any great degree of success until Kahn, studying the factors affecting precipitation with syphilitic serum, worked out a test embodying optimum conditions for precipitation. The conditions established are as follows:

1. Optimum concentration.

2. Instability of antigen-saline suspension.

3. Serum antigen suspension proportion

4. Agitation.

The rate and degree of precipitation is markedly affected by concentration of the ingredients. Excessive dilution of the serum with salt solution, or of the antigen with alcohol or salt solution will so delay the precipitation reaction that incubation at higher temperatures is necessary. On the other hand, excessive concentration, especially of the beef heart extractives in the antigen may actually inhibit the precipitation reaction. If the ingredients are employed at certain optimum concentrations the precipitate is formed almost immediately without incubation and thus the contamination that so often accompanies incubation is eliminated.

When the antigen-saline mixture consists of very fine highly dispersed particles (an opalescent mixture) the precipitation reaction is not as marked as when the antigen-saline mixture contains relatively large particles. If, on the other hand, the particles in suspension are not readily dispersed when brought in contact with saline or serum, the suspension cannot be used in a diagnostic test since particles would be found in the serum-antigen suspension mixture with both negative and positive serum. In other words a coarse readily dispersable antigen-saline suspension is demanded by the condition of this test. This

necessitates the use of a standard controlled method of preparing the antigen-saline suspension.

Experiments indicate that for complete precipitation in any given serum-antigen suspension, there must exist a proper quantitative relationship between the reacting substances of the serum and the reacting substances of the antigen. A given volume of a strongly potent serum will react best with a relatively large amount of antigen suspension (as 1 part serum to 1/3 part of antigen suspension), whereas the same volume of a weakly positive serum will react best with a smaller volume of antigen suspension (as 1 part of serum to 1/12 part of antigen suspension). Therefore it appeared that in order to be successful a precipitation test should include varying proportions of serum and antigen. This principle is used by Kahn in his standard procedure which consists of a 3-tube test, the technique of which will be described later.

It was discovered by Kahn in his early work that agitation definitely increased the precipitates in the weakly positive serums. Although shaking is not necessary in strongly potent serums it does render them more easily read and is always employed in the regular procedure. The effect of shaking was studied by Kahn, and optimum conditions for the rate of shaking and for the length of time were worked out.

THE KAHN TEST

Preparation and standardization of antigen. Kahn antigen is an alcoholic extract of dehydrated powdered beef heart, the preparation of which is, in brief, as follows: Fifty grams of powdered beef heart are extracted with 200 cc. of ether for ten minutes and filtered. Three subsequent ether washings are made with 150 cc. of ether respectively, using the same extraction period of 10 minutes. After the last filtration, the beef heart is spread out on paper and dried until every trace of ether odor is removed. The beef heart is now weighed (it will be found that approximately 5 grams of beef heart will be lost during the process of extraction with ether) and 5 cc. of 95% alcohol is then added to each gram of the dry beef heart, and allowed to extract in the dark at room temperature for three days. At the end of the three day extraction period the beef heart is separated from the alcoholic extract by filtration and discarded. The extract is now ready for cholesterolization. 0.6% of pure cholesterol is added to the extract. The dissolving of the cholesterol is facilitated by gently rotating the flask containing the mixture in warm water. After the cholesterol is thoroughly dissolved the antigen is filtered to render it free from foreign particulate matter. The antigen is

then ready to be titrated. The purpose of the titration is to determine the smallest amount of physiological salt solution which will produce a suspension capable of redissolving upon the further addition of saline or negative serum.* It is desirable (but not necessary) to obtain an antigen which has a titer of 1 cc. antigen plus 1.1 cc. of physiological salt solution.

Antigens prepared from dried beef heart by the standard method will frequently vary in sensitiveness due to variations in the biological product employed. A certain degree of antigen sensitiveness was selected as standard on the basis of clinical and serological studies carried out jointly by the Michigan Department of Health and the Department of Dermatology and Syphilology at the University of Michigan Hospital.

Standardization. If an antigen prepared according to the required method is not identical in sensitiveness with standard antigen as shown by its reactions with syphilitic serum, the antigen must be modified in sensitiveness in such a way that it will correspond with the standard. The correction method involves an increase or decrease of the concentration of beef heart

* Solution of the suspension particles as a preliminary step also takes place in the case of positive serum. It is, however, almost immediately succeeded by reprecipitation, which does not occur in the case of negative serum or of salt solution.

extractives of the antigen, or the addition to the antigen of an extract known as sensitizing reagent, especially prepared from ether extractives of beef heart.

Preparation of serum. Serum used in the Kahn test must be clear and free from particles. Therefore the specimen of blood must be either centrifugalized or allowed to stand for some time in order that the clear serum may be separated from the clot and cells. The serum after being heated for 30 minutes at 56°C. in a water bath, is ready to be tested. If serum is to be kept several hours after heating before being tested, a subsequent heating period of 10 minutes at 56°C. should be applied. Serums when allowed to stand for some time at ice box temperatures tend to become cloudy. This cloudiness usually disappears when the serum is reheated for 10 minutes.

Routine diagnostic test. The routine Kahn test is a 3-tube procedure in which three different proportions of serum and antigen suspension are mixed. Into tubes 1, 2 and 3, supported in a metal rack are measured respectively, 0.05 cc., 0.025 cc. and 0.0125 cc. antigen suspension. To each of the three tubes is added 0.15 cc. of previously heated serum. The rack of tubes containing the antigen suspension and serum is shaken for three minutes at about 280 oscillations per minute. To the first tube containing 0.05 cc. antigen suspension is added 0.1 cc. saline. To the second and third tubes containing 0.025 cc. and 0.0125 cc. of antigen suspension is added 0.5 cc. saline.

Reading and interpretation of results. The negative reactions appear opalescent but free from visible precipitate. The positive reactions are marked by the formation of precipitates which may be very fine and barely visible to the unaided eye (±, doubtful reactions) or may show increasingly marked precipitates up to a well defined precipitate suspended in a clear medium (++++, four plus reactions). The intermediate precipitates are graded +, ++ and +++, respectively. The following scheme summarizes the steps of the procedure:

	Tube 1	Tube 2	Tube 3
Serum:Antigen Suspension	3:1	6:1	12:1
Antigen Suspension, cc.	0.05	0.025	0.0125
Serum, cc.	0.15	0.15	0.15
Saline, cc.	1.0	0.5	0.5

Quantitative procedure. In the quantitative procedure, antigen suspension is mixed in the proportion of 1 to 15 with increasing dilutions of serum. The serum dilutions are made according to the following scheme.

Dilution No.	Dilution Ratio.	
(1)	1	= undiluted serum
(2)	5	= 0.2 cc. undiluted serum plus 0.8 cc. normal saline.
(3)	10	= 0.7 cc. of (2) plus 0.7 cc. normal saline.
(4)	20	= 0.2 cc. of (3) plus 0.2 cc. normal saline.
(5)	30	= 0.2 cc. of (3) plus 0.4 cc. normal saline.
(6)	40	= 0.1 cc. of (3) plus 0.3 cc. normal saline.
(7)	50	= 0.1 cc. of (3) plus 0.4 cc. normal saline.
(8)	60	= 0.1 cc. of (3) plus 0.5 cc. normal saline.

Eight tubes are placed in a rack and 0.01 cc. antigen suspension is pipetted into each tube. The serum dilutions in 0.15 cc. amounts are then added to the tubes containing the antigen suspen-

sion, pipetting from the highest to lowest dilution. The rack of tubes is shaken 3 minutes, 0.5 cc. salt solution is added to each tube and the results read. The routine 3-tube test previously described does not differentiate the potency of two, 4-plus serums, yet one serum may contain a much greater number of reacting units than the other. The quantitative test was designed to measure relatively the potency of a serum which is strongly positive. The relative potency expressed in Kahn reacting units may be computed according to the formula S = 4 D, S representing serum in terms of reacting units, and D representing the highest serum dilution ratio in which there is a definite precipitate. Thus, if 1:5* represents the highest dilution of serum capable of producing a precipitate, 4 x 5 or 20 reacting units are contained in this serum. Likewise if 1 : 40 represents the highest dilution in which a serum gives definite precipitation the serum contains 4 x 40 or 160 units. In the quantitative test a definite precipitate (++++, +++ or ++) is considered as positive, and a weak precipitate (+ or ±) as negative.

Additional procedures include quantitative and qualitative tests with spinal fluids, tests with fluids from syphilitic lesions and micro tests to be used only when minute amounts of serum or spinal fluid are available.

* Serum dilution ratio is taken as the ratio of the volume of diluted serum at a given dilution to the volume of the serum before dilution. Thus if a serum is diluted 1 to 5, the serum dilution ratio is 5.

REVIEW OF PREVIOUS STUDIES ON THE HEATING OF SERUM.

As has already been mentioned the heating of serum within certain limits markedly increases its sensitiveness. Relatively few quantitative studies have been carried out on this important phase of the Kahn procedure. A brief summary of the work that has been done follows.

In Kahn's original studies he found that serum heated for 30 minutes at 56°C. gave much stronger reactions than did a portion of the same serum unheated. In a series of 177 tests all of which gave either a +++ or ++++ reaction when the serums were heated for 30 minutes at 56°C., only 48 gave equally strong reactions when the unheated serums were used.

An experiment to determine the length of the heating period of serum at 56°C. which would give the best results were carried out as follows. Unheated serums were divided into 6 parts, 1 part of each serum was allowed to remain unheated and the remaining parts were heated at 56°C. for 5, 10, 20, 30 and 60 minutes, respectively, after which they were tested by means of the regular procedure. It was shown that the more potent the serum the shorter the heating period required. Strong serums did not require any heating while the somewhat less potent ones required only five minutes. In general there was no difference in potency between serums heated for 60 minutes and those heated for 30 minutes

except for a slight increase in sensitiveness in the ones heated for 60 minutes. It was found that there was no drop in sensitiveness of the serums after 90 minutes heating and but a slight drop in sensitiveness after 3 hours.

Other experiments were carried out at temperatures higher than 56°C. and longer than 30 minutes. A number of serums were heated 30 minutes at 58°C., samples withdrawn the remaining portion of each serum heated at 62°C., samples being withdrawn and tested after 30, 60 and 120 minutes, respectively. The additional heating at 62°C. caused only a slight fall after 30 minutes, but became quite appreciable after 60 minutes and very pronounced after 120 minutes.

Kendrick* made some interesting observations on the relation between the heating of serum and sensitiveness of the reaction in Wassermann and Kahn tests. A number of positive serums were examined by both tests before being heated and also examined after being heated at 56°C. for varying periods of time. In the case of the Wassermann test the most sensitive results were, in general, obtained with the unheated serums. Serums heated for 30 minutes were about equal in sensitiveness to the unheated serum. A sudden continuous drop in sensitiveness was observed after 30 minutes heating. In the Kahn test, on the other hand, the sensitiveness was least with the unheated serums and rose steadily as the heating period was increased. The sensitiveness of the Kahn test was less than that of the Wassermann

*See reference (3) in the Bibliography.

test when the unheated serums were used. Wassermann and Kahn tests gave similar results when the heating period was 30 minutes, and the Kahn test was more sensitive for longer heating periods.

In some unpublished studies from Kahn's laboratory, Nagle and Mc Dermott carried out three series of experiments on the heating of serums, in which they compared the effect of heating serum for various periods of time at 60°C., 58°C., and 45°C., with that of standard heating treatment for 30 minutes at 56°C., the results of which follow.

In the first series of tests 15 serums were divided into 4 portions which were heated as follows. The first portion heated at 56°C. for 30 minutes served as a control. The second portion was heated at 60°C. for 10 minutes, the third and fourth portions at the same temperature, but for 15 and 20 minutes, respectively. The sensitiveness of the serums were comparable to that of the control when the heating at 60°C. lasted 10 minutes; it was less than that of the control when the heating lasted 15 and 20 minutes. In the second series serums were heated at 58°C. for 10, 15 and 20 minutes and it was found that they were less sensitive than that of the control. In the third series, serums were heated at 45°C. for 15 and 30 minutes and gave much less sensitive reactions than the control.

Present Experiments on the Heating of Serums.

The present studies on the heating of serum were prompted largely by the growing interest in, and the practicability of, the Kahn test. In these studies both the 3-tube and quantitative procedures, previously described, were used in a somewhat modified form. Instead of the regular 3-tube test, a 1-tube test was employed using a serum-antigen suspension ratio of 12, which is the same as that used in the back tube of the regular test. Since only small amounts of individual serums were available, it was necessary to reduce the amounts of serum and antigen suspension to one-half of that which is ordinarily used in the 12:1 serum-antigen suspension proportion. Thus, .006 cc. of antigen suspension was pipetted to the bottom of Kahn tubes by means of a 0.2 cc. pipette graduated in .001 cc. To the antigen suspension was added .075 cc. of serum also with a 0.2 cc. pipette. The tests were shaken in the standard way, 0.05 cc. of saline added and the results read.

The quantitative procedure used was according to standard requirements except that one-half the usual amount of serum, namely, 0.1 cc. was used in the preparation of the serum dilutions, and due to the small amount of serum available, dilution ratio 1 was not employed. The serum dilutions were prepared according to the following scheme.

Dilution No.	Dilution Ratio.	
1	5	0.1 cc. undiluted serum + 0.4 cc. salt solution.
2	10	0.3 cc. of dilution 1 + 0.3 cc. salt solution.
3	20	0.2 cc. of dilution 2 + 0.2 cc. salt solution.
4	30	0.1 cc. of dilution 2 + 0.2 cc. salt solution.
5	40	0.1 cc. of dilution 2 + 0.3 cc. salt solution.
6	50	0.2 cc. of dilution 3 + 0.3 cc. salt solution.
7	60	0.2 cc. of dilution 5 + 0.1 cc. salt solution.

Standard antigen was used throughout these studies. The serums used in these experiments were carefully removed by means of a bulb pipette from the clots after sufficient amounts had been withdrawn for the routine diagnostic test for syphilis in the laboratories of the Michigan Department of Health. The numbers given the specimens in the laboratory were used in this experiment in order to facilitate checking our technique. The serums were then examined for cells or foreign particles. Those which were badly hemolized or showed material which could not be removed were considered undersirable and discarded. Each serum was divided into 2 portions. One portion was heated for 30 minutes at 56°C. and served as a control. The other portion was heated at a higher temperature for varying periods of time. The serums were kept stoppered during the heating period in order to prevent evaporation.

Experiment No. 1. Nine hundred fifty individual serums were used in this experiment. The portion of the serum serving as a control was heated for 30 minutes at 56°C., while the other portion was heated for 10 minutes at 60°C. A qualitative 1-tube procedure was employed in making the tests, and

the results recorded on a 4-plus basis in Table I. Identical results were obtained in 915 (96.3%) of the serums tested. The results obtained from the other 35 serums showed variations ranging from + to +++. The data revealed that in only 11 (1.1%) serums, was there a difference of more than +. With 6 of these 11 serums the control showed more sensitive reactions than those heated for 10 minutes at 60°C. while the remaining 5 gave more sensitive reactions when heated for 10 minutes at 60°C.

It appears from this experiment with serum tested by means of the qualitative 1-tube procedure, that a heating period of 10 minutes at 60°C. is equivalent to the regular heating period of 30 minutes at 56°C.

Experiment No. II. The same heating treatment, 10 minutes at 60°C., was used with 65 serums in a second series of experiments in which the quantitative procedure was used. The results obtained are listed in Table 2. There was agreement in 59 (91%) of the serums. In the other 6 (9%) serums the variations never exceeded the difference between the number of Kahn units given by one dilution, and the number that would be given by the next higher or next lower dilution. This experiment thus confirms the results of the previous experiment.

Experiment No. III. Prompted by the satisfactory results obtained with the serums heated for 10 minutes at 60°C. a series of tests was undertaken using a 5 minute heating period and a temperature of 62°C. The 1-tube qualitative method was employed. The results as obtained from 260 serums are presented in Table III. In 245 (94.2%) of the serums, identical results were obtained. The remaining 15 (5.8%) gave variations ranging from + to +++. Twelve of the serums which showed variations gave more sensitive reaction in the control, while the remaining 3 gave more sensitive reaction when heated for 5 minutes at 62°C. Although most of the serum in this experiment gave the same results when the heat treatment was 5 minutes at 62°C., yet we find that the majority of the ones showing variations gave more sensitive results when heated according to the standard method. This suggests that 62°C. may be higher than the optimum heating temperature for serum. However, had other time periods at 62°C. been tried, better results might have been obtained.

Experiment No. IV. In this experiment one portion of each of 35 serums were heated for 3 minutes at 65°C. and the remaining portion of each serum heated according to the standard method. The results observed are listed in Table IV. Comparable results were observed in only 27 (77%) of the serums. Less sensitive results were shown in 7 (20%) of the serums. In only one serum was the sensitiveness greater when heated for 3 minutes at 65°C. than when heated according to the standard method.

Experiment No. V. A few serums were heated for 3 minutes at 68°C. and comparison made as usual with the control. None of the serums were coagulated when subjected to this heating treatment. The results are recorded in Table V.

From experiments III, IV and V, it appears that a temperature of 68°C. is not sufficiently high to destroy all the serum reacting substances and thus prevent precipitation with antigen but, on the other hand, it appears that most satisfactory results are obtained when the heating temperature is below 62°C.

Experiment No. VI. An experiment was carried out to determine the relative sensitiveness of a serum heated at 56°C. for varying time periods. Several four plus serums were pooled for this study. The pooled serum was poured into individual vials which were placed in a water bath at 56°C. At various intervals a vial was removed and its contents tested immediately with the quantitative procedure. The shortest heating period was 10 minutes, and the longest was 300 minutes. The results are plotted in Figure I, in which the ordinates represent serum sensitiveness in Kahn units, and the abscissae represents duration of the heating period in minutes. Its sensitiveness was increased by the 10-minute heating period and became consistently greater as the heating period of 40 minutes was reached. Longer heating periods decreased the sensitiveness, but even the maximum heating period tried, 300 minutes, did not reduce

the sensitiveness to that of the unheated serum.

Experiment No. VII. A number of vials containing portions of a given lot of pooled serums were placed in a water bath at 60°C. At stated times a vial was withdrawn and the serum tested with the quantitative procedure. The results are represented graphically in Figure 2. It appears from this experiment that serums when heated at 60°C. reach their maximum sensitiveness in a very short time, the optimum heating period in this case being between 2 and 14 minutes. If the heating period is prolonged beyond 14 minutes a fall in sensitiveness is observed, and when the heating period is prolonged beyond 30 minutes for the serums tested the sensitiveness is less than that of the unheated serum.

Experiment No. VIII. In order to determine the persistence of the increase of sensitiveness brought about by heating, the following experiment was carried out. Equal portions of a given lot of pooled serum were poured into a number of vials and placed at 60°C. At given intervals 3 vials were removed; the contents of one of these vials was used for an immediate test (quantitative procedure) and the contents of the other 2 vials were similarly tested after having stood at ice box temperature for 5 minutes and at room temperature for 1 hour, respectively. The results are represented graphically in Figure 3.

This experiment would indicate that the increase in sensitiveness due to heating is destroyed when the heated serum

is allowed to stand at room temperature for an hour before being tested, and that the increase is reduced when the serum is placed at 7°C. before being tested. It would thus appear that a heated serum gives its maximum sensitiveness when tested within a short time after receiving the heat treatment. It must be remembered that this experiment was carried out with but a single lot of pooled serum and that hence the results may not apply in all cases. Since the presistence of the heating effect on the sensitiveness of serum is of practical importance in the laboratory, further experiments are planned to test the general applicability of this observation.

CONCLUSIONS

1. It appears from this study that a heating treatment of serum for 10 minutes at 60°C. gives practically the same results as the regular heating period of 30 minutes at 56°C., irrespective of whether the serums are tested with the qualitative 1-tube procedure or with the quantitative procedure.

2. Heating temperatures of 62°C. and over are found to be less satisfactory in the Kahn procedure with serums than heating temperatures of from 56°C. to 60°C.

3. Serums heated at 60°C. appear to reach their maximum sensitiveness in from about 2 to 10 minutes while serums heated at 56°C. require a much longer period.

4. Serums remaining at room temperature for one hour or at 7°C. for 5 minutes after being heated 10 minutes at 60°C., give less sensitive reactions than when tested immediately.

TABLE I

EFFECT OF DIFFERENT HEATING PERIODS OF SERUM ON SENSITIVENESS OF KAHN REACTIONS.

No. of Serums	Reaction after heating for 30 minutes at 56°C.	Reaction after heating for 10 minutes at 60°C.
144	++++	++++
3	++++	+++
1	++++	+
1	+++	++++
11	+++	+++
4	+++	++
3	+++	±
3	++	++++
2	++	+++
5	++	++
2	++	+
2	++	±
1	+	++
2	+	+
1	+	±
7	±	±
6	±	-
2	-	++
4	-	±
746	-	-

TABLE II

EFFECT OF DIFFERENT HEATING PERIODS OF SERUMS ON SENSITIVENESS OF QUANTITATIVE KAHN REACTIONS.

No. of Serums	No. of Kahn units after heating serum for 30 minutes at 56°C.	No. of Kahn units after heating serum for 10 minutes at 60°C.
1	280	280
2	240	240
1	200	200
1	160	160
3	120	120
1	*120	80
1	*80	40
8	40	40
3	*20	40
1	*20	4
13	20	20
30	4	4

* Variations.

TABLE III

EFFECT OF DIFFERENT HEATING PERIODS OF SERUM ON SENSITIVENESS OF KAHN REACTIONS.

No. of serums	Reaction after heating for 30 minutes at 56°C.	Reaction after heating for 5 minutes at 62°C.
23	++++	++++
1	++++	+++
1	++++	++
1	++++	+
1	++++	±
1	+++	++++
2	+++	+++
4	+++	++
1	+++	±
1	++	++++
2	++	++
1	++	+
1	++	±
1	+	+
1	±	±
1	±	-
1	-	+
216	-	-

TABLE IV

EFFECT OF DIFFERENT HEATING PERIODS OF

SERUM ON SENSITIVENESS OF KAHN REACTIONS.

No. of Serums	Reaction after heating for 30 minutes at 56°C.	Reaction after heating for 5 minutes at 65°C.
11	++++	++++
1	++++	+++
1	++++	±
2	+++	+++
3	+++	++
4	++	++
1	++	±
1	+	++
2	+	+
3	+	±
1	+	-
1	±	±
4	-	-

12-2-27V

Fig. 1

Potency of serum heated at 56°C. for different time periods.

Fig. 2

Potency of serum heated at 60° C. for different time periods.

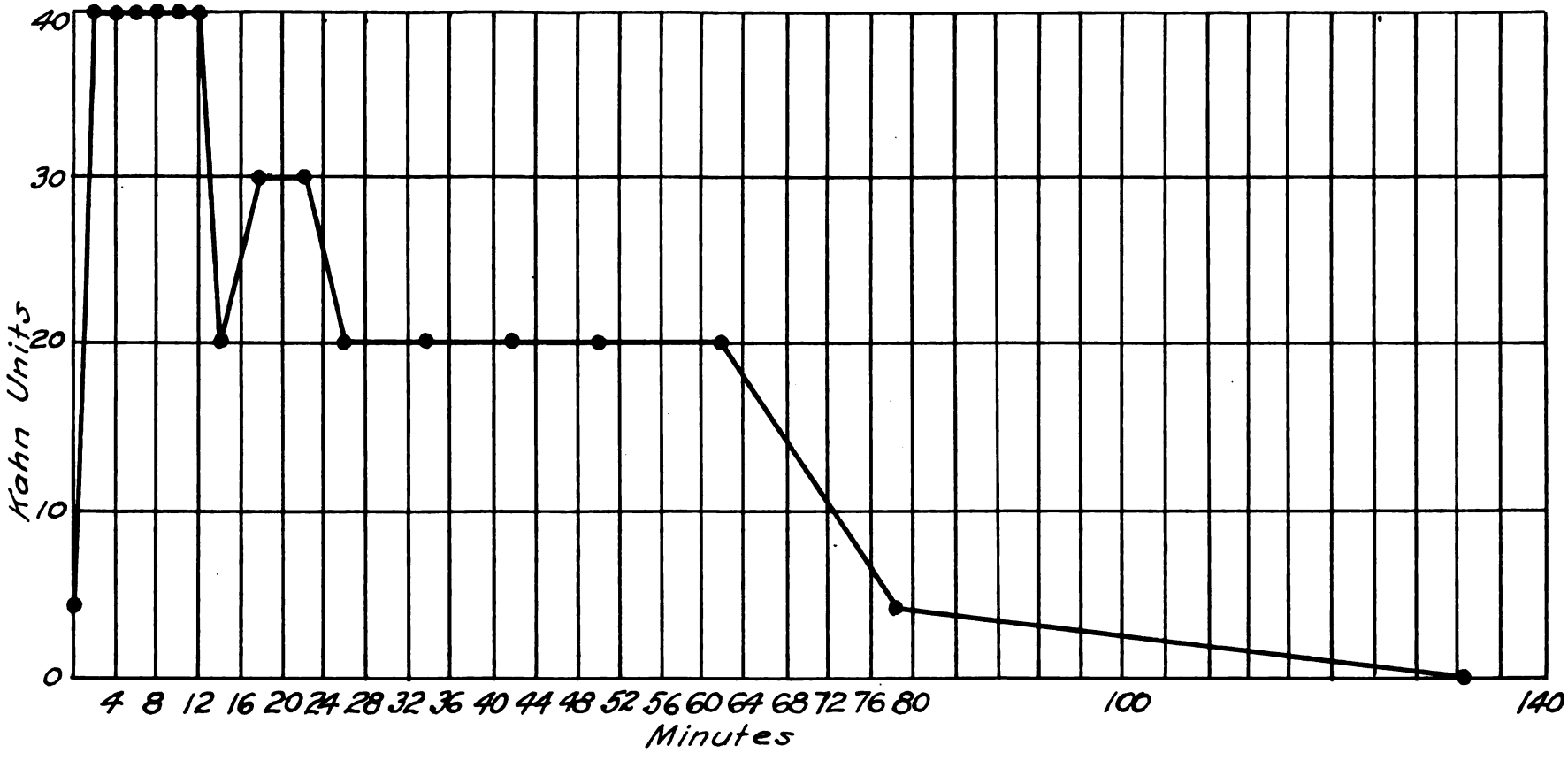
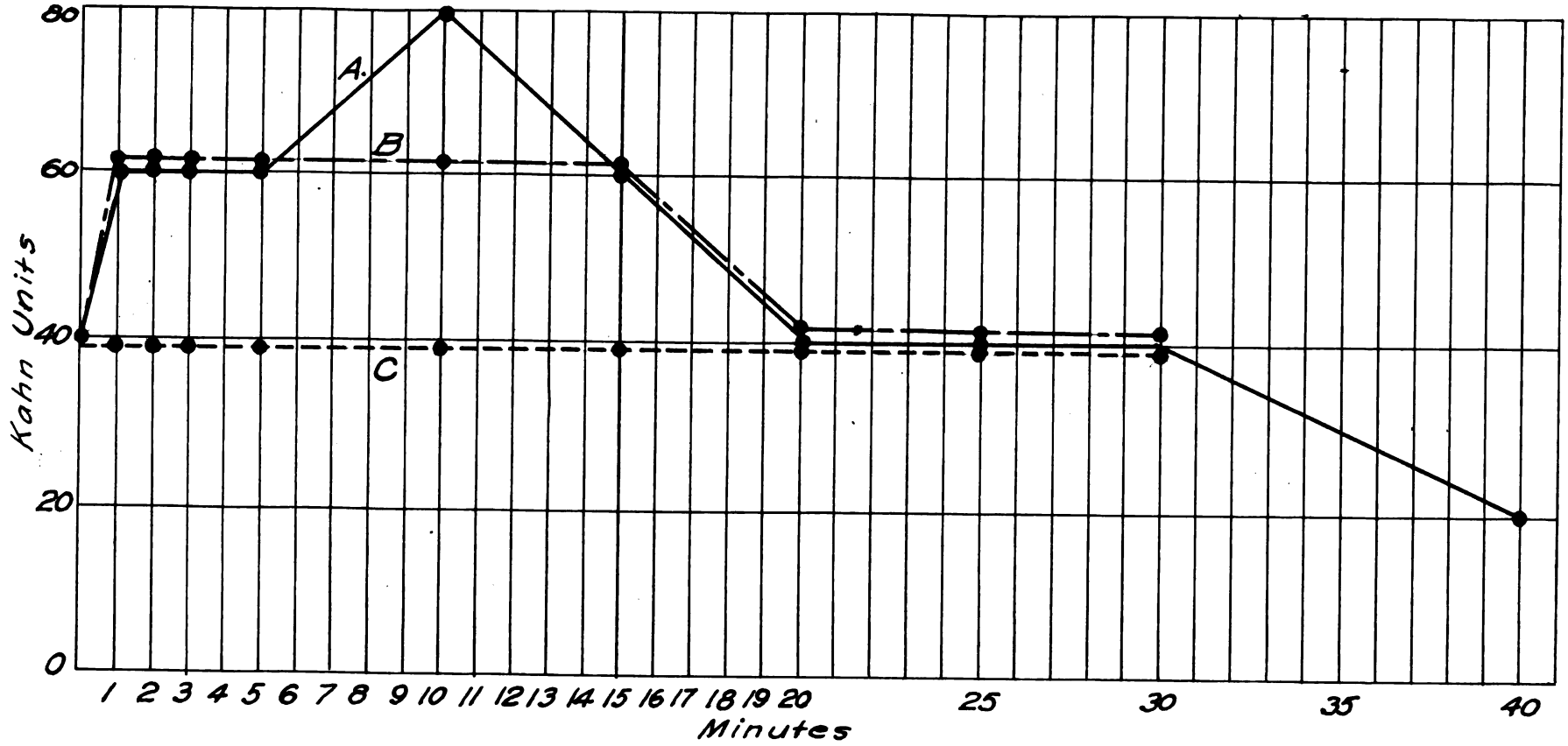


Fig. 3

Potency of serum heated at 60°C. for different time periods.



Legend—

——— A. Serum tested immediately after heating period.

——— B. Serum tested after a 5 min. period at 7°C. following the heating period.

------ C. Serum tested after standing one hour at room temp. following the heating period.

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BIBLIOGRAPHY

(1) Kahn, R. L.: Serum Diagnosis of Syphilis by Precipitation. 1924.

(2) The Kahn Test - A Practical Guide. 1928. (in manuscript)

(3) Kendrick, P. L.: Comparative Heat Resistance of Serum in the Kahn and Wassermann Tests. Reprint Series, Michigan Department of Health, December, 1925.

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