STUDIES IN AGGLUTINATION USING BACTERIUM ABORTUS AS ANTIGEN

Thesis for Degree of M. S.

Harold Kenyon Archbold

1925

THESIS

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Submitted to the Faculty of the Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of Master of Science.

By

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June 1925.

THESIS

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Studies in Agglutination Using *Bacterium Abortus* as Antigen.

Introduction.

The agglutination test has generally been accepted as a practical means of diagnosing bovine infectious abortion by most investigators, although a few prefer the complement fixation test. The latter claim that the agglutination test is too variable in its reaction, while the complement fixation test is more delicate and specific, and the reagents which enter into the reaction are more easily controlled. Recent work, comparing the results of the two tests, show that they agree very closely.

At the present time a different technic for the agglutination test is employed in almost every laboratory, which in all probability causes a considerable degree of variation in the results obtained. A study of the various factors which effect the agglutination test and their subsequent solution will lead to a much more reliable method of diagnosing bovine infectious abortion, and consequently make the test more acceptable to all concerned.

Within recent years much time and effort have been devoted by investigators to the detection and formulation of distinct immunological types among the members of a given species of bacteria, the members of which are culturally identical or nearly so. The purpose of this study is to determine the existence of different immunological types of *Bacterium abortus*, and the possibility of an existing difference in agglutinability, (both of which would effect the reliability of the test and result in a marked difference in the reading of the results by different laboratories) by means of cross agglutination and

agglutinin absorption tests. Also to determine the agglutinability of antigens of different hydrogen ion concentration.

Review of Literature.

Kolmer (1) states that, "Metchnikoff, Charin, and Rogers had noticed peculiarities in the growth of Bacillus pyocyaneus, when cultivated in immune serum, which we now believe were due to agglutinins, Gruber and Durham and Bordet(1894 - 1896) were the first to recognize that the agglutination reaction was a separate function of immune serum. While investigating the Pfeiffer phenomenon of bacteriolysis with Bacillus coli and the cholera vibrio, these investigators found that if the respective immune serums were added to bouillon cultures of these two species, the cultures would lose their turbidity, flake-like clumps would form and sink to the bottom of the tube, and the supernatant fluid would become clear. Gruber at the same time called attention to the fact that agglutinins were not absolutely specific for their own antigen, but would agglutinate, to a lesser extent, closely allied species of bacteria.

"In 1896 Pfaundler drew attention to a peculiar phenomenon observed when bacteria were grown in an immune serum. Long, and more or less interlaced threads of bacteria developed, which were regarded as due to agglutinins. At that time considerable emphasis was laid upon the importance of Pfaundler's reaction, but at present the ordinary agglutination tests have superseded this reaction as a practical diagnostic procedure.

"In 1896 Widal and Grünbaum first turned these facts to practical use in the diagnosis of typhoid fever. These investigators found that the serum of patients suffering from typhoid fever acquires

a high agglutinating power for bacillus typhosus, and since this phenomenon generally manifests itself comparatively early in the disease, its recognition has considerable diagnostic importance. It is purely accidental that we speak of the 'Widal Reaction' in typhoid fever, rather than of the 'Grünbaum Reaction', for the latter observer conducted similar studies independent of Widal, but, owing to a lack of patients, Widal preceded him in a publication of a more extensive work.

"At the present time this diagnostic reaction is known as the Gruber-Widal reaction. It has proven of great value to a large number of different investigators, not only in making the serum diagnosis of typhoid fever, but in other infections as well".

The agglutination test for the diagnosis of infectious abortion in cattle was probably first used in 1907-1908, by a Danish veterinarian, Grinstead (2). His results, however, were not published until near the end of 1909. Grinstead showed that the blood of cows which had aborted, agglutinated the abortion bacilli in higher dilution than the blood of normal animals.

McFadyean and Stockman (3) were the first to demonstrate that the agglutination test was of considerable diagnostic value in the abortion disease. Very shortly afterwards Holth published similar preliminary observations upon this test. The former investigators concluded from their rather meager data that, "having regard to what has been the experience in other diseases for which the test has been extensively used, the difference between the agglutinating power of a serum of a cow affected with abortion, and that of some normal animals, is too slight to inspire confidence in the test". Later these men reported favorably upon the agglutination reaction and its value as a diagnostic test in infectious abortion. In this

report (4) they state, "In conclusion it may be said that the agglutination test has now passed beyond the probationary stage, and deserves to be adopted as the method of diagnosis which is most generally applicable in suspected cases of contagious abortion. The discovery of such a reliable method of diagnosis removes what has hitherto been the greatest obstacle in grappling successfully with the disease either by private effort or under state regulation. It may also be noted that it provides an easy means of ascertaining whether there is any real foundation for the view that other organisms than the abortion bacillus, discovered by Bang, are responsible for abortion on an epizootic scale".

Mohler and Traum (5) made a comparative study of the value of the two serological tests and recommended the agglutination test, due to its simplicity and the saving of time, except in doubtful cases, when the complement fixation test may be employed to advantage.

Seddon (6) pointed out several very important factors in connection with the agglutination test for infectious abortion. He is of the opinion that this test is not simply a matter of dilution, but a quantitative reaction. This same fact has been reported on by several other investigators.

Connaway (7) states, "The serological tests, agglutination and complement fixation, when properly applied, are as reliable for the diagnosis of infectious abortion in cattle, as tuberculin is for the detection of tuberculosis".

Williams (8) expresses himself as follows, "The frequency of abortion in a herd usually corresponds fairly well with the intensity of infection with Bacterium abortus. In those herds where abortion

and other interferences with reproduction occur, the agglutination and complement fixation test usually show intense infection with Bacterium abortus. But such reactions can only show that Bacterium abortus is somewhere active or has been active, and cannot show that it caused a given abortion".

Fitch (9) after making single agglutination tests on all animals in several different herds and checking the histories of the animals with his tests, reaches the following conclusion , "At present the results of the agglutination test cannot be utilized as a basis for control measures for abortion disease".

Birch and Gilman (10) commenting on the three statements above state, "If these views represent , as we believe they do, a fairly reliable cross-section of opinion as it exists in regards to the value of the serological tests in the practical handling of infectious abortion it is evident that there are yet fundamental considerations on which all are not agreed, nevertheless there are many well established facts which are quite universally accepted and to which additions are being made".

In a later paper on further studies with the agglutination test, Fitch (11) states that it is the most reliable of the serological methods of diagnosing infectious abortion. He says further, "It will indicate the amount of herd infection, although it will not pick out individual aborters. A positive reaction to this test means past or present infection".

Rettger (12) believes that the agglutination and complement fixation tests may be satisfactorily applied in the diagnosis of infectious abortion. Their constancy and reliability make them indispensable in the study and control of this disease.

According to Williams (13) "In the diagnosis of contagious abortion the most practical method without sacrificing animals is presumably by the agglutination or complement fixation tests. In the application of these tests to abortions occuring in clinics such as the ambulatory clinic of the New York State Veterinary College and others, combined with cultural searches and guinea pig inoculations from the abort and the membranes, the presence of Bacterium abortus is recognizable in about 50% of cases. But in many of these, other bacteria are also present. What relation, if any, they have to the abortion is not known".

Schroeder (14) states that "If the agglutination test for bovine infectious abortion is or can be made serviceable to distinguish with certainty between safe cows which, though they have been exposed to infection, and may have aborted, and may show some reaction, and dangerous carriers of abortion bacilli, it would tend greatly to reduce the difficulties that must be overcome in attempts to suppress the abortion evil through the use of sanitary measures".

It appears from the foregoing review of literature that the present day investigators are gradually accepting the agglutination test as a valuable aid in the diagnosis of bovine infectious abortion and they have come to look upon it as almost indispensable.

Of recent years many investigators have attempted to separate strains of an organism into different and definite groups by means of the agglutination and agglutinin absorption tests. Some of these investigations have given valuable information on the subject while the results obtained by others have been fruitless. Without a doubt these efforts, in some instances, have served a very useful purpose, but Torrey (15) thinks that we may extend this method of grouping in such a manner as to depart from a natural system of classification. He states

that a type or group, if established upon a rational basis, should represent a distinct serological entity.

By the use of the agglutinin absorption test, Hermanies (16) showed that gonococcus could be separated into six distinct heterologous types. The agglutinins produced by strains of one type could not be absorbed by strains of another. In five of these types, the agglutinins were always bound by the strains belonging to the same type. The antigenic complex of the strains forming a type seemed to have a similar constitution, Strains forming type 2, however, varied in their agglutinogenic and absorptive capacity, for, while a number of strains bound the agglutinins produced by some strains completely, the binding capacity in others was more or less limited and variable, and in a few entirely absent.

Warren (17) reported that the agglutination test does not serve to differentiate strains of gonococcus into serological groups.

Torrey (18) reports, "An analysis by agglutinin absorption methods of the serological relationships of 77 gonococcus strains, isolated from cases of acute and chronic gonorrhea and its complications, indicated that they may not be distributed among a number of clear-cut immunological types. Straight agglutination tests have yielded almost no evidence as to specific serological relationships".

Fleming and Clemenger's (19) results indicate that the B. influenza of Pfeiffer is really an associated group of bacteria without the marked unity in its immunity reaction as is seen in other epidemic causing bacteria. Their results "show that almost every strain as regards to agglutination is a law unto itself".

Small and Dickson (20) were able to separate nine strains of B.influenza into four distinct groups. Their work was based upon

cross-agglutination and checked by the agglutinin absorption test.

Bell(21) maintains that the influenza bacillus represents a heterologous group of organisms as shown by cross-agglutination and agglutinin absorption tests and that identical strains do occur.

Using cross agglutination, agglutinin absorption, and protective tests with strains isolated from different localities, Roes (22) reports that the various strains of B. influenza apparently do not differ in kind.

Maitland and Cameron (23) studied 38 strains of B. influenza isolated from /_hospital patients during a period when no cases of epidemic influenza were seen and found that nearly all the strains possess a serological individuality as determined by agglutination and agglutinin absorption tests".

Rivers and Kohn (24), working with 13 meningitic strains of influenza bacillus, found that eleven were culturally alike and fell into two groups by the agglutinin absorption test. Seven strains fell into group 1. Three strains fell into group 2, with one intermediate group. The other two strains stood alone culturally and serologically.

Fousier and Myer (25) performed a series of absorption tests with formalinized suspensions of Bact. abortus and Bact. melitensis which led to a fourfold grouping of 14 Bact. abortus and Bact. melitensis strains. The grouping revealed these principals:

1. "An antiserum cannot be exhausted by strains of another group".
2. " A strain acts in a uniform manner(qualitatively) on all strains in another group under the same absorbing conditions."
3. "Strains within the same group do not necessarily act in a uniform manner on one another when absorbed from the same antiserum".

Evans (26) reports, "The agglutinin absorption test with 49 strains of Brucella meletensis has shown:

"1. This species may be differentiated into at least seven serological groups. Four of these groups include only one or two strains each, and are relatively unimportant.

"2. The majority of bovine and porcine strains fell into one large group (30strains) which is designated variety abortus. Two strains of human origin were in this variety. Two of the small serological groups are so closely related to this one that they should be considered as subvarieties.

"3. Simple agglutination tests can not differentiate between varieties, abortus and melitensis".

Kliger (27) found a oiture of a typical B. coli and B. dysentery Shiga, which yielded immune sera possessing agglutinating properties of practical equal quantity for each other. Absorption experiments made with their respective sera indicated that two distinct agglutinins were produced in about equal amounts in the process of immunization of rabbits. The agglutinins were specific ones, each for its culture, and accessory agglutinins left the specific agglutinins quantitatively unaffected.

Avery (28) found that the biological classification of pneumococcus distinguishes four distinct groups. He based these types upon well defined immunological differences.

Hooker (29) found that strains of B. typhosus could be placed into three fairly well defined groups. He based his classification upon the complement fixation and agglutinin absorption tests.

Technic Employed.

Preparation of Sera: The sera were obtained from thirty rabbits each of which had previously been injected with a suspension of a different strain of living Bacterium abortus. Each strain was grown for twenty-four hours at 37°C on a slant of beef liver agar. The growth was suspended in sterile physiological salt solution and diluted until turbidity corresponded with tube 5 of McFarland's nephelometer. Immediately after preparation one cubic centimeter of the suspension of the organisms was injected into the marginal ear vein of the rabbit. Each of the rabbits used had previously reacted negative to the agglutination test using a strain of Bact. abortus as antigen. Three weeks after injection, the rabbits were bled from the ear in order to determine the titre of the serum. The titre in each case proved sufficiently high enough for the study. The rabbits were then bled from the heart, the blood allowed to clot at room temperature, placed in the ice box over night, centrifuged on the following day, and the clear serum removed and placed in the ice box until used.

The same procedure was followed in building up sera for the agglutinin absorption tests. In this case only eight strains of Bacterium abortus were used.

Cross-Agglutination Test: Ten tubes were used in the cross agglutination test. Two c.c. of salt solution was placed in the first tube and one c.c in the remaining nine tubes. Two-tenths c.c. of serum was added to the first tube, giving a dilution of 1-10. One c.c. of this mixture was transferred to the second

tube, giving a dilution of 1-20. This process was carried through the remaining tubes and one c.c. of the mixture in the last tube was discarded, thus diluting the original serum one-half on each transfer. To each of the ten tubes one c.c. of the antigen was added, which again resulted in diluting the serum one-half in each tube and decreasing the turbidity of the antigen one-half. The final dilutions were: 1-20, 1-40, 1-80, 1-160, 1-320, 1-640, 1-1280, 1-2560, 1-5120, and 1-10240. The tubes were incubated at 37°C and readings taken at the end of 24 and 48 hours. The thirty sera were run against each of the thirty antigens thus giving cross-agglutination of homologous and heterologous strains.

Preparation of Antigens for Cross Agglutination and Agglutinin Absorption Studies: Thirty strains of Bacterium Abortus were grown for 24 hours at 37°C on liver agar slants as recommended by Huddleson(30). The growth of four slants was washed off with phenolized salt solution, pH 6.8, and the suspension made up to correspond with tube number one of the McFarland's nephelometer, and stored in dark brown bottles in a cool place.

Agglutinin Absorption Test: The bacterial growth from 25 liver agar slants was washed off with 20 c.c. of phenolized salt solution. To the suspension, 20 c.c. of homologous serum (diluted 1-5) was added thus giving a dilution of 1-10. This was incubated at 37°C for two hours and placed in the ice box over night. The suspension was centrifuged the next day and the clear supernatant fluid, which in reality is the serum diluted 1 to 10, pipetted off. And agglutination test was run on the serum, using the homologous antigen, to see if all the homologous agglutinins had been removed. If there was any degree of

agglutination, the serum was reabsorbed in the following manner. The growth from five slants was washed off with the phenolized salt solution, and the suspension concentrated by centrifugation. The supernatant fluid was discarded and the sedmented organisms added to the 1 to 10 serum. This was incubated as before and centrifuged. This method of reabsorption was carried on until the agglutination test showed that the homologous agglutinins had all been removed. Then the absorbed serum was run against 25 heterologous antigens and the results recorded.

The agglutination test used in this work is the same as that used above except that eachbf the tubes contained one c.c. of salt solution and one c.c. of the absorbed serum was placed in the first tube. The method of making progressive dilutions was the same. The resulting dilutions were: 1-40, 1-80, 1-160, 1-320, 1-640. Only five tubes were used as it was thought that this would give a satisfactory end point, which proved to be true in each case. The tubes were incubated at 37°C and readings taken at the end of 24 and 48 hours. Only the 48 hour readings are recorded here as they were approximately the same.

Preparation of Colorimeter: It was necessary to prepare a colorimeter which would have a range of pH from 4.0 to 8.8 with a difference of a .4 pH between each tube. A prepared tablet for each pH required was placed in a tube containing 10 c.c. of distilled water and dissolved. The following indicators were added to the corresponding tubes: Tubes pH 4.0 and 4.4 eight drops of Brom Phenol Blue were added, tubes of pH 4.8, 5.2, 5.6 eight drops of Methyl Red were added, tubes of pH 6.0, 6.4, 6.8, 7.2 and 7.6 eight drops of Brom Thymol Blue were added, and for tubes of pH 8.0, 8.4, and 8.8 eight drops of Cresol Red were added. A crystal of Thymol was added to each tube as a preservative. This colorimeter

is used as a standard to prepare solutions of corresponding hydrogen ion concentration.

Preparation of Antigens of Different pH: Strain 2a was selected for this study. Antigens were prepared with the following pH values: 4.0, 4.4, 4.8, 5.2, 5.6, 6.0, 6.4, 6.8, 7.2, 7.6, 8.0, 8.4, and 8.8. Preliminary tests had been carried out on 10 c.c. of distilled water to determine the number of drops of N/1 HCl and N/1 NaOH it took to obtain a buffered solution which would correspond to each of the above pH values on the colorimeter. A capillary pipette was used throughout the work for the HCl and one for the NaOH, in order that the drops would be uniform. 200 c.c. of each buffered solution was made up using the above data as a basis, and each solution was checked with the colorimeter. A 24 hour beef liver agar slant growth of strain 2a was washed off with each of the buffered solutions. The turbidity of each was made to correspond with tube number one of McFarland's nephelometer. The suspensions were checked to determine their value and stored until ready for use.

Agglutination Test: Eight tubes were used in the test. 2 c.c. of the antigen was added to each tube. The serum was added to each tube in the following amounts:

1st tube..... .08 c.c. of serum, giving the dilution 1 to 25
2nd tube..... .04 c.c. of serum, giving the dilution 1 to 50
3rd tube..... .02 c.c. of serum, giving the dilution 1 to 100
4th tube..... .01 c.c. of serum, giving the dilution 1 to 200
5th tube..... .08 c.c. of 1 to 20 serum, giving the dilution 1 to 500
6th tube..... .04 c.c. of 1 to 20 serum, giving the dilution 1 to 1000
7th tube.....02 c.c. of 1 to 20 serum, giving the dilution 1 to 2000
8th tube..... .01 c.c. of 1 to 20 serum, giving the dilution 1 to 4000

Seven sera was used, including two negative and five positive. The tubes were incubated at 37°C and readings taken at the end of 24 and 48 hours. The latter only is recorded here as both readings were approximately the same.

TABLE I.

History of Strains of Bact. Abortus Employed in these Studies.

Strain	Date of Isolation	Origin
1	Unknown	Received from B.A.I. prior to 1915
2	1915	Aborted fetus (herd A, Mich.)
2a	1910	Calif. Exp. Stat. Aborting sow.
3	1915	Aborted fetus (herd A, Mich.)
6	1915	Udder of cow 995(Abortion Exp.herd)
7	Unknown	In laboratory prior to 1916.
16	June, 1917	Aborted fetus (near Owosso, Mich)
20	1919	Placenta of cow following abortion. (near Ubly, Mich.)
22	Sept., 1919	Aborted fetus (herd A, Mich)
60	Feb. 1920	Udder of cow (herd A, Mich)
100	June, 1920	Udder of cow (abortion exp.herd)
200	Jan., 1920	Udder of cow (abortion exp. herd)
300	1922	Aborted fetus, Michigan.
400	1922	Aborted fetus, Michigan.
805	May, 1919	Placenta of cow following abortion (abortion exp. herd)
1659	1923	Udder of cow (abortion exp. herd)
2010	Rec'd 1920	Dr. Graham, Illinois University.
Berlin	Unknown	Obtained in Germany 1919.
C	March 1917	Aborted fetus (herd A, Mich.)
D1	1923	Aborted fetus near Detroit.
E	Prior to 1916	Unknown.
England	Unknown	Royal Veterinary College April 1919
G.B.Swine	1920	Aborted fetus of sow(near Lansing,Mich)
H16	1922	Aborted fetus, Kansas Exp.Station.
H321	1924	Aborted fetus (nutrition Exp.herd).
J	1916	Aborted fetus (herd B, Mich)
King	Unknown	Obtained 1923 from Dr. Stockman,England.
L	December 1920	Aborted fetus (herd A, Mich.)
Roadard	Unknown	Purdue University, 1924.
W	Unknown	Wisconsin University 1915

Experimental Data.

Cross Agglutination: During the last decade many investigators have attempted to group strains of an organism by the use of cross agglutination. A review of the literature shows that some very encouraging results have been obtained, but failures also have been recorded.

In this work, thirty strains of Bacterium abortus were used in the production of thirty sera. Thirty different antigens were prepared from the same strains, and an agglutination test was performed with each serum against each of the antigens. Charts from 1 to 30 inclusive show the results of these tests, each chart representing the results of agglutination of one serum against the thirty antigens. The black columns show the end point of agglutination at the end of 24 hours, while the red columns denote the end point of agglutination at 48 hours.

It may be seen that antigen H221 is agglutinated only by its homologous serum and by heterologous serum 2a. There were seven sera whose agglutinability was variable. These seven were H221, 805, J, D_1, 7, 1, and 100. The variation was quite marked in several instances.

The agglutinability of the antigen was not constant for each serum. No line could be drawn to setoff any one group of strains, unless it be antigen H221. Taking the results of agglutination of 29 of the antigens, excluding H221, one will note that the average end point of agglutination is about 1 to 1280. There were some antigens which were agglutinated by a serum in a dilution as high as 1 to 10240 and as low as 1 to 40. Chart 31 denotes the average agglutinability of each of the antigens against all of the sera. Two antigens differ from the remain-

ing antigens very distinctly. H 221 has the lowest average of all. The average agglutinability of Roadard is much lower than that of the remaining 28 strains.

Agglutinin Absorption: In addition to the use of cross agglutination in attempting to classify strains of a certain organism, much work has been done with the agglutinin absorption test in this respect. Better results have been attained with the latter, although failure has been common.

In this work eight of the original strains were used to produce sera. They were, H221, 805, 400, 2a, 2010, Roadard, Swine 1, and W. Each strain was absorbed by its homologous strain, and to make sure that all of the homologous agglutinins were taken out, an agglutination test was run on the absorbed serum using its homologous antigen. A negative test showed complete absorption of the homologous agglutinins. If a positive test was obtained the serum was reabsorbed until a negative test was obtained. When the serum was proved to be absorbed agglutination tests were run against 25 heterologous antigens. Serum 400 was absorbed also by the heterologous antigen 2a, and then the absorbed serum was run against the same antigens.

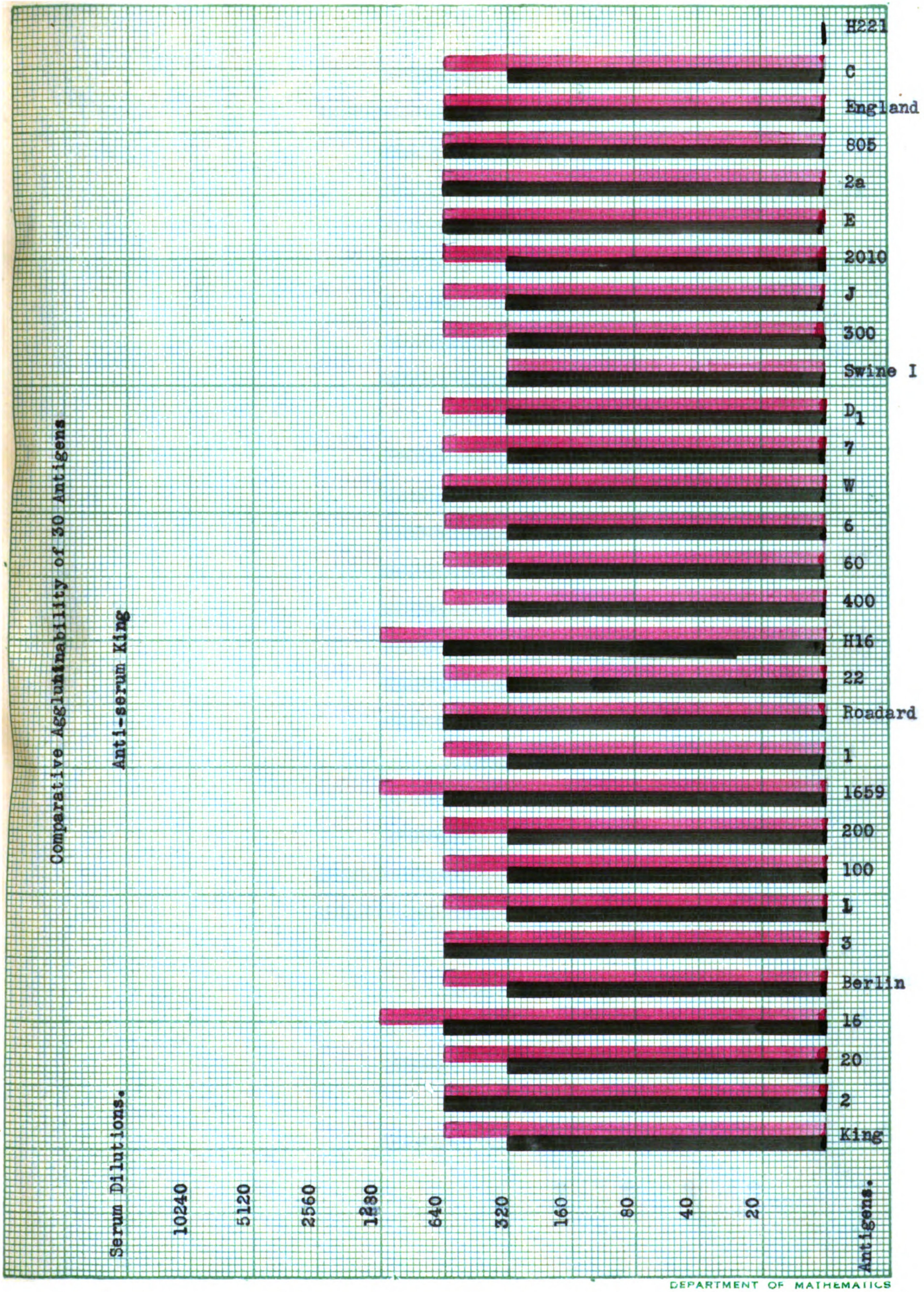
Tables 2 and 3 give the results of these tests. The results were recorded as P(partial), + (positive), -(negative), and 0 (no test).

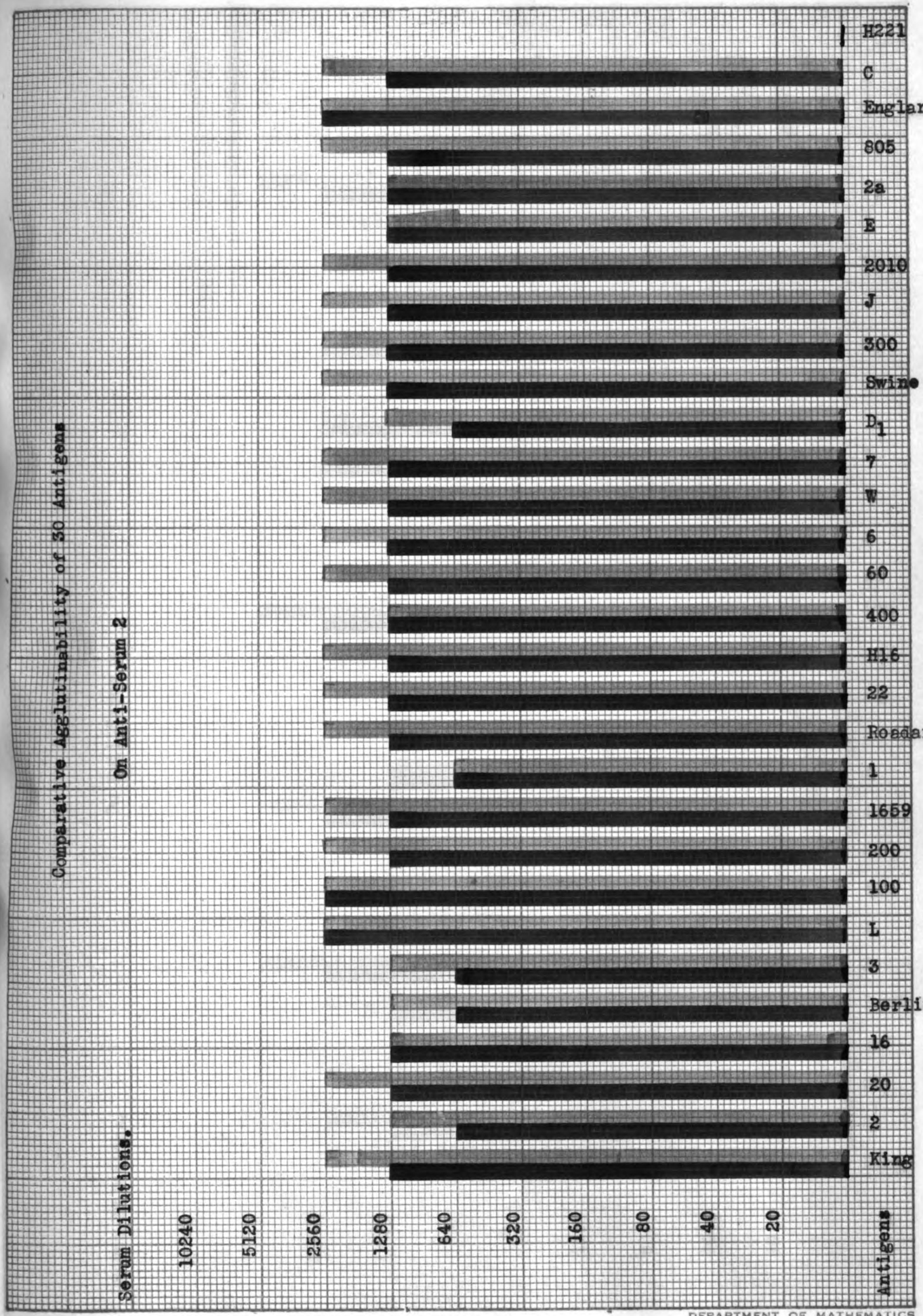
Strain 400 is the only antigen which absorbed completely all the agglutinins from its homologous serum. Swine 1 left agglutinins in for one strain. 2010 absorbed all agglutinins except for two strains. 805 and W left agglutinins for three strains. The remaining tests show that after absorption of the homologous agglutinins, there remained agglutinins for a majority of the heterologous strains.

The Effect of the Hydrogen Ion Concentration of the Antigen Upon The Agglutination Test: Many laboratories vary as to the pH of the antigen used in their agglutination test. Table 4 gives the results of the agglutination tests using antigens of different pH values.

The only marked difference shown is on the antigens of a pH 4.0, 4.4, and 4.8. In these, there is a tendency for the acid antigen to cause the formation of a flocculent precipitate in the presence of serum, which settles to the bottom of the tube. It has the appearance of NH_4OH to which alum has been added. The occurrence of this phenomenon was constant in tubes of pH 4.0, and 4.4, and in two sera it occurred with antigen of a pH 4.8. It occurred in the negative serum as well as in the positive.

Chart 1


Comparative Agglutinability of 30 Antigens
Anti-serum King
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens.
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-Serum 2
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
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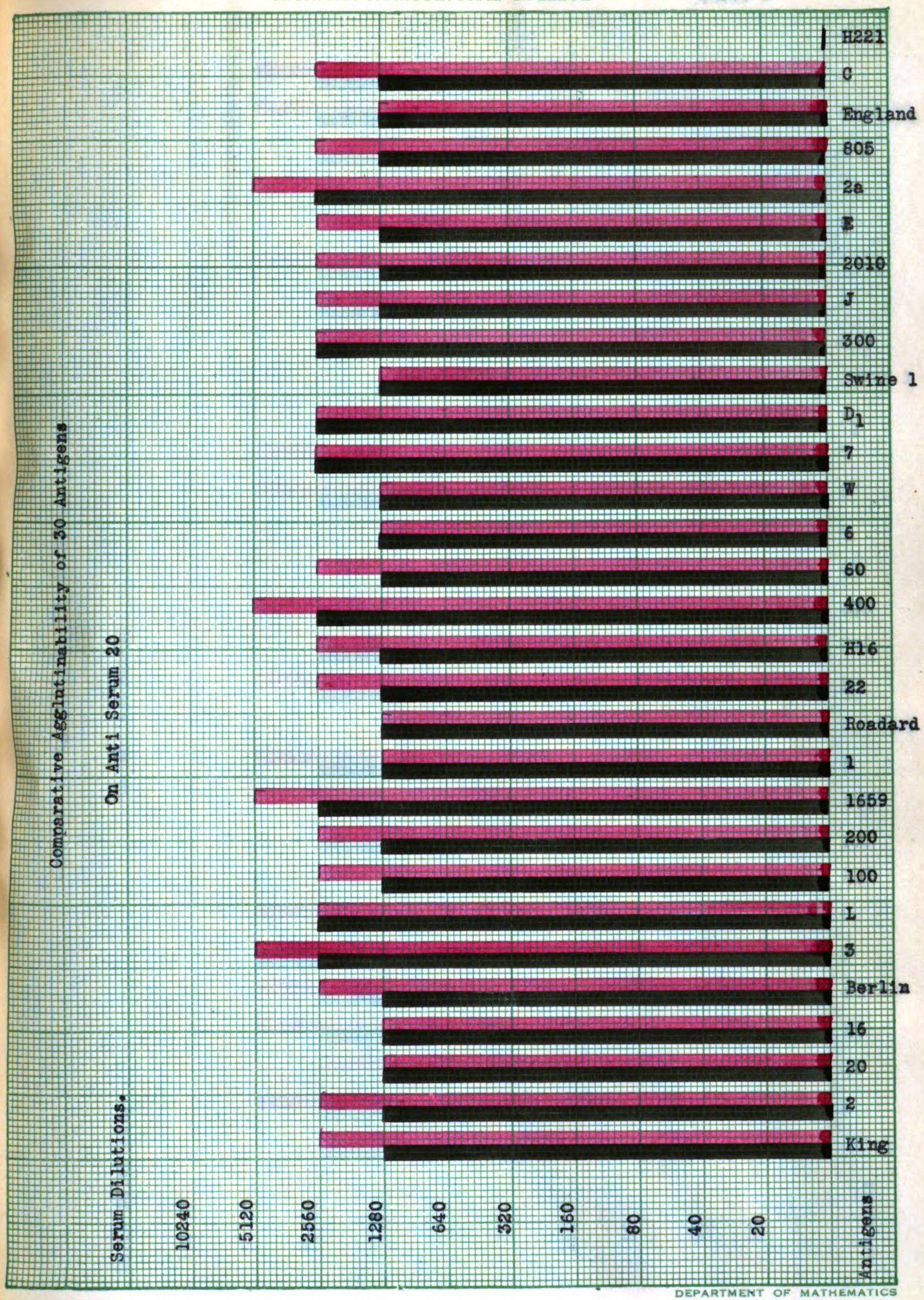
King


Comparative Agglutinability of 30 Antigens
On Anti Serum 20
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
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2010
J
300
Swine 1
D1
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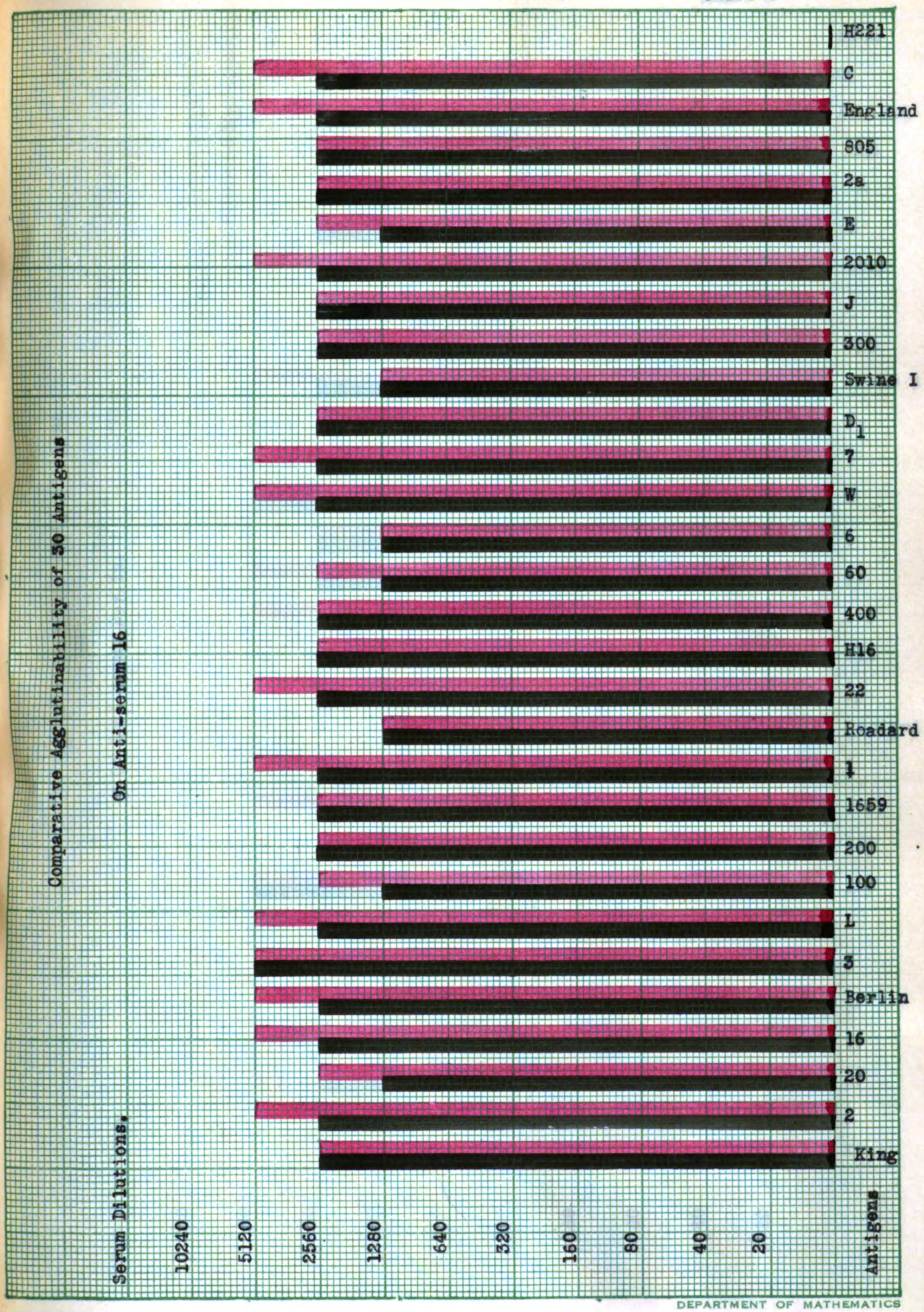
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-serum 16
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
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2010
J
300
Swine I
D1
7
W
6
60
400
H16
22
Roadard
4
1659
200
100
L
3
Berlin
16
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King


Comparative Agglutinability of 30 Antigens
On Anti-serum Berlin.
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens.
H221
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Swine 1
D1
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400
H16
22
Roadard
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1659
200
100
L
3
Berlin
16
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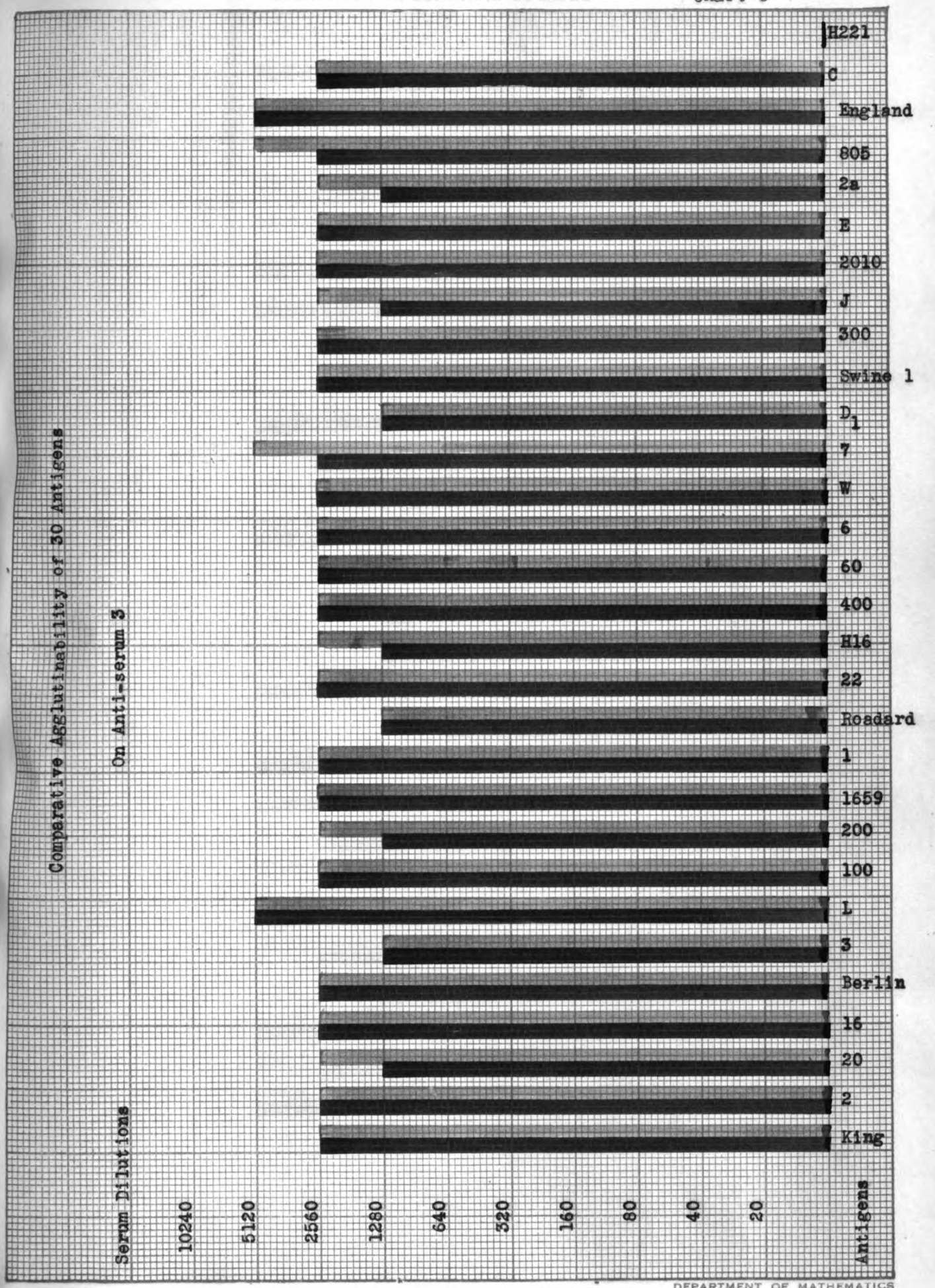
2
King


Comparative Agglutinability of 30 Antigens
On Anti-serum 3
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20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
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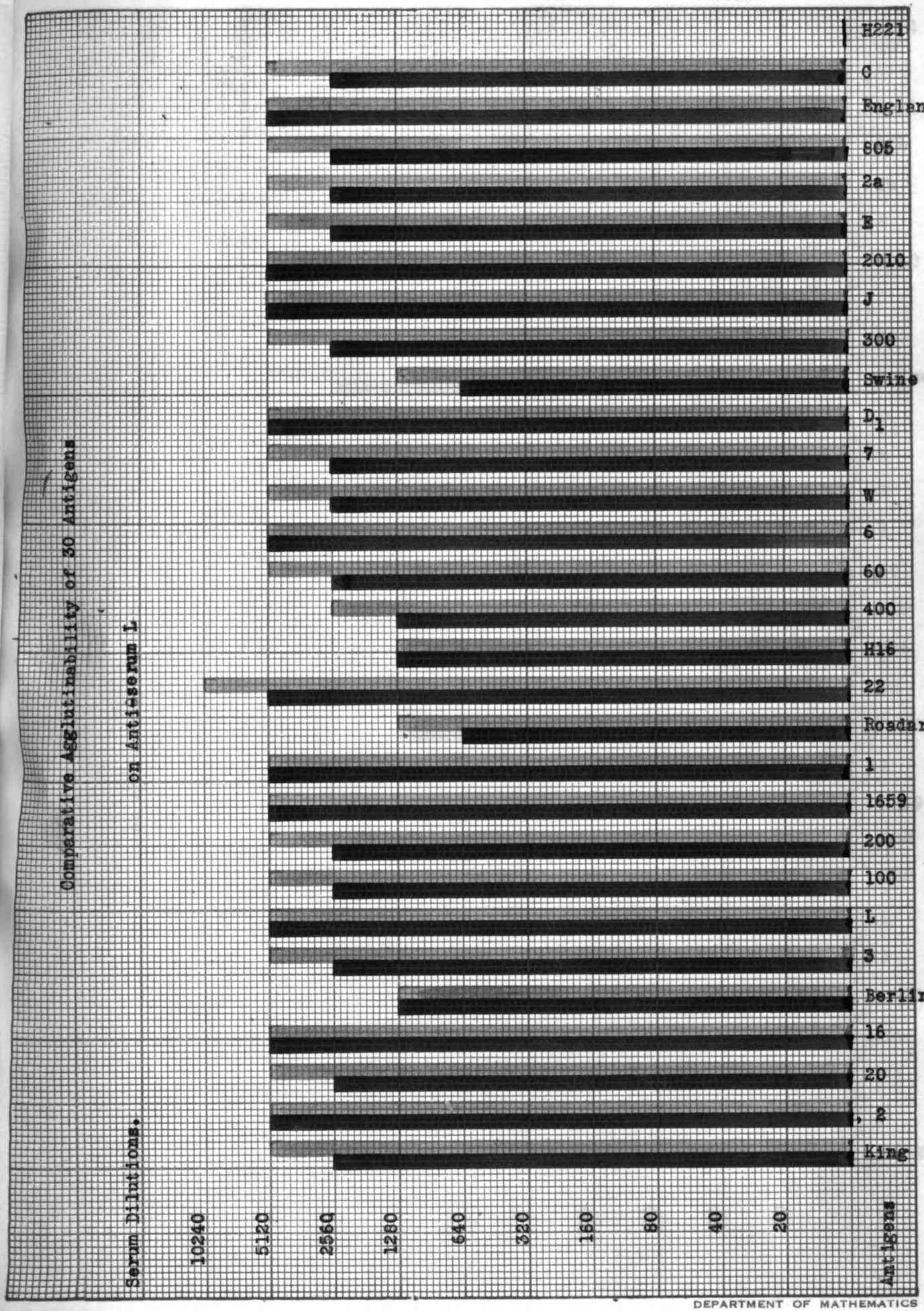
H16
22
Rosdard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Antiserum L
Serum Dilutions.
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1280
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Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
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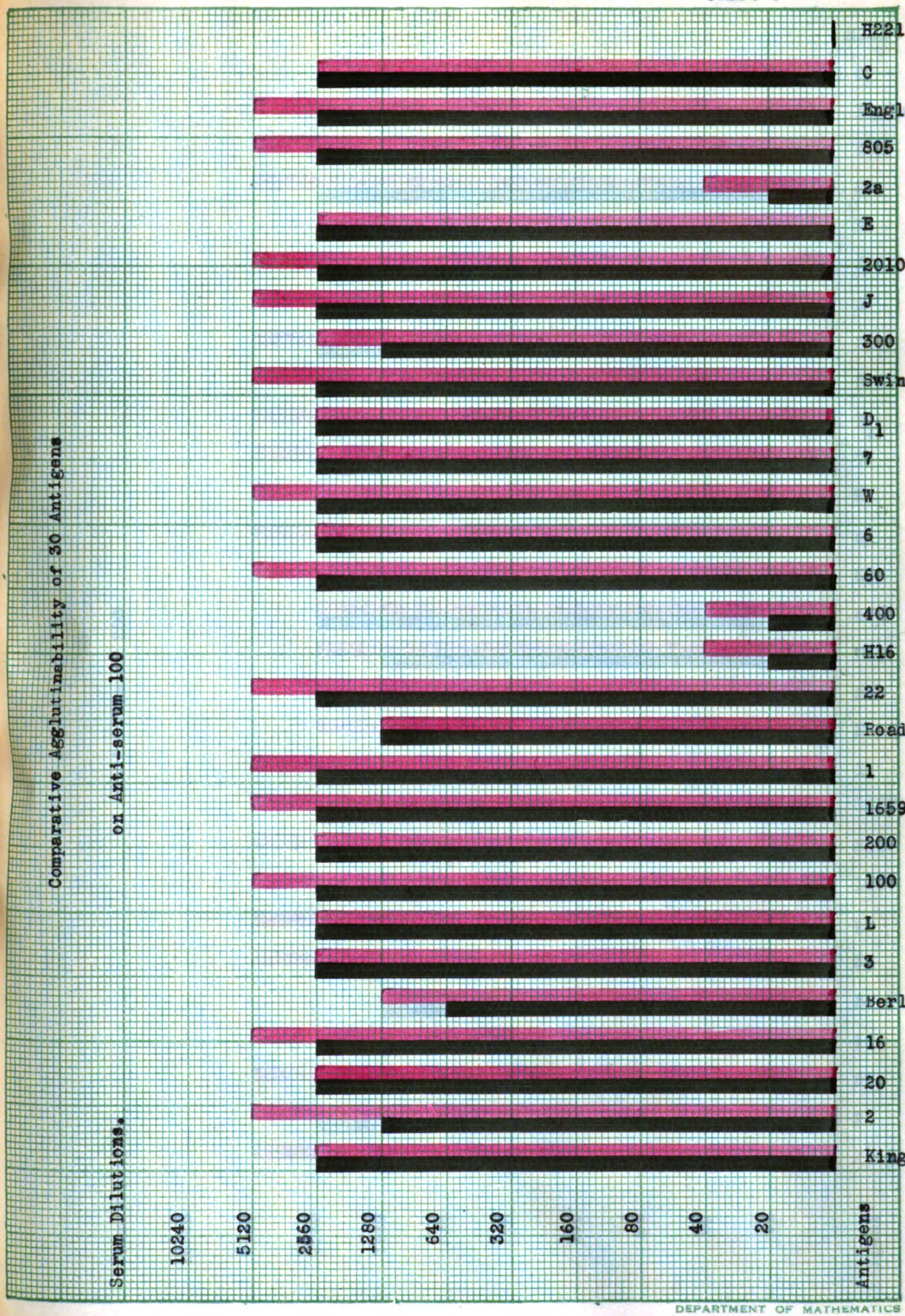
400
H16
22
Rosdard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
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Serum Dilutions.
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5120
2560
1280
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160
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40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
7
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6
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400
H16
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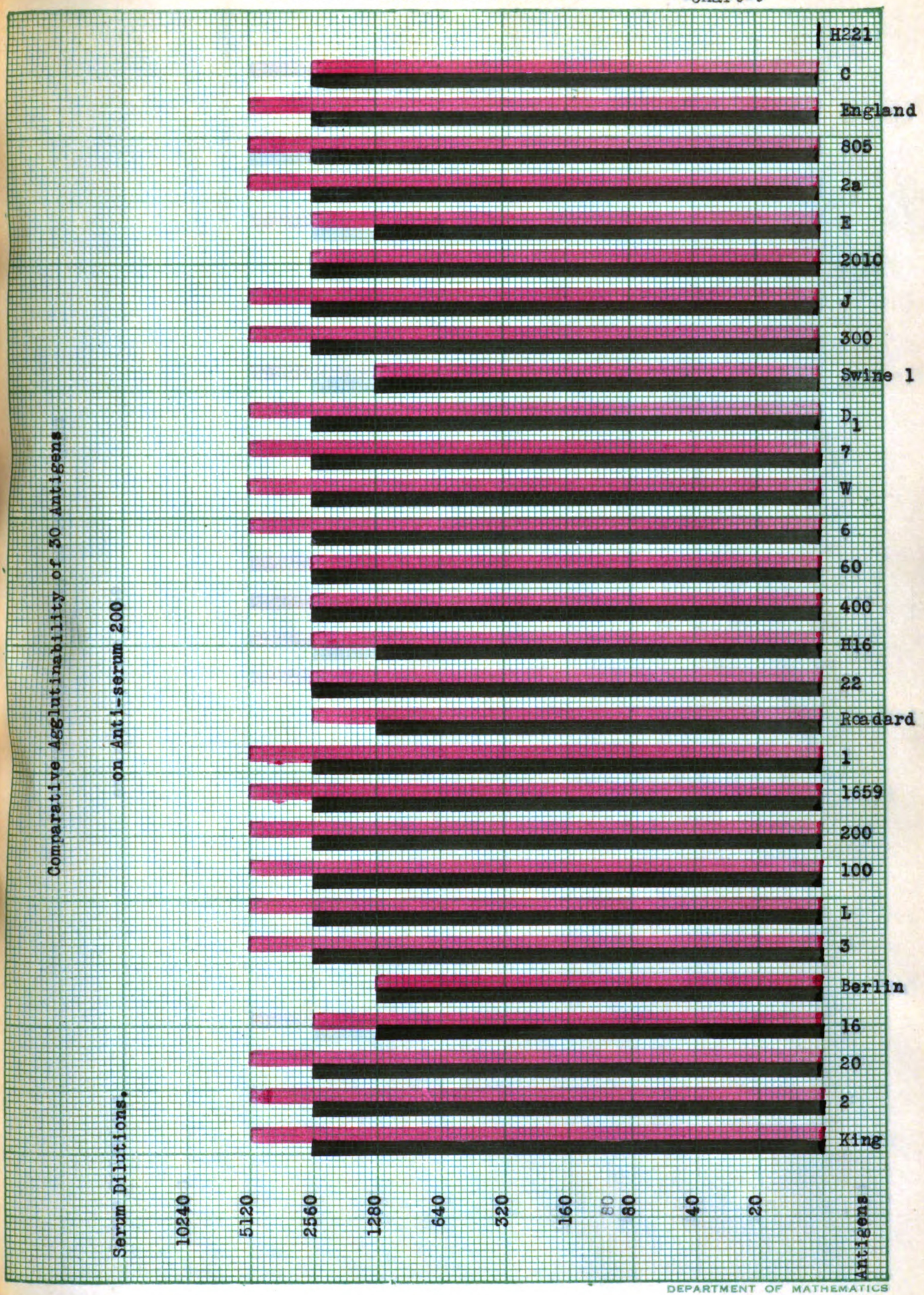
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
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Serum Dilutions.
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40
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Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
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400
H16
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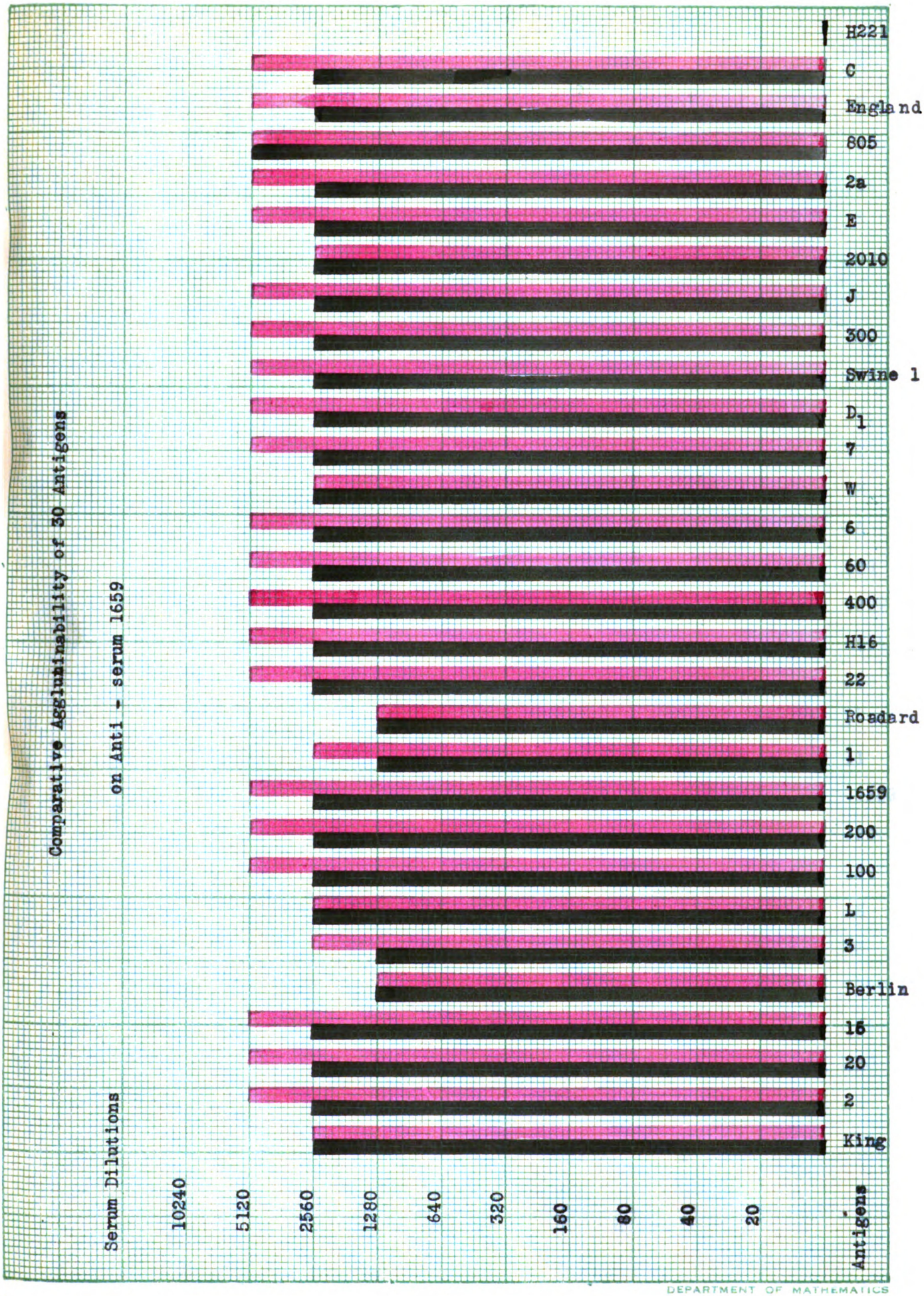
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
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Serum Dilutions
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Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
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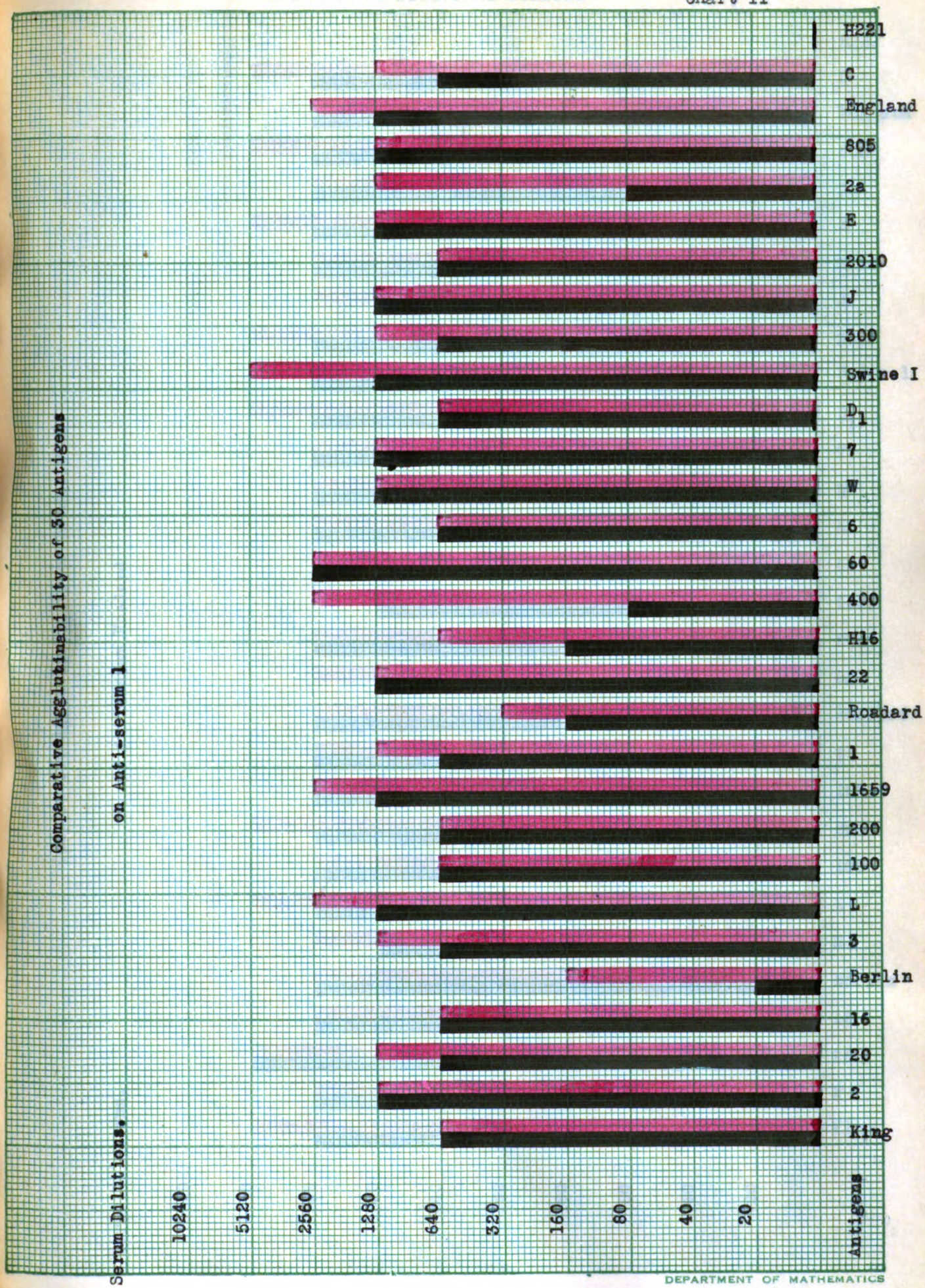
400
H16
22
Rosdard
1
1659
200
100
L
3
Berlin
15
20
2
King

Chart 11


Comparative Agglutinability of 30 Antigens
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Serum Dilutions.
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160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
7
W
6
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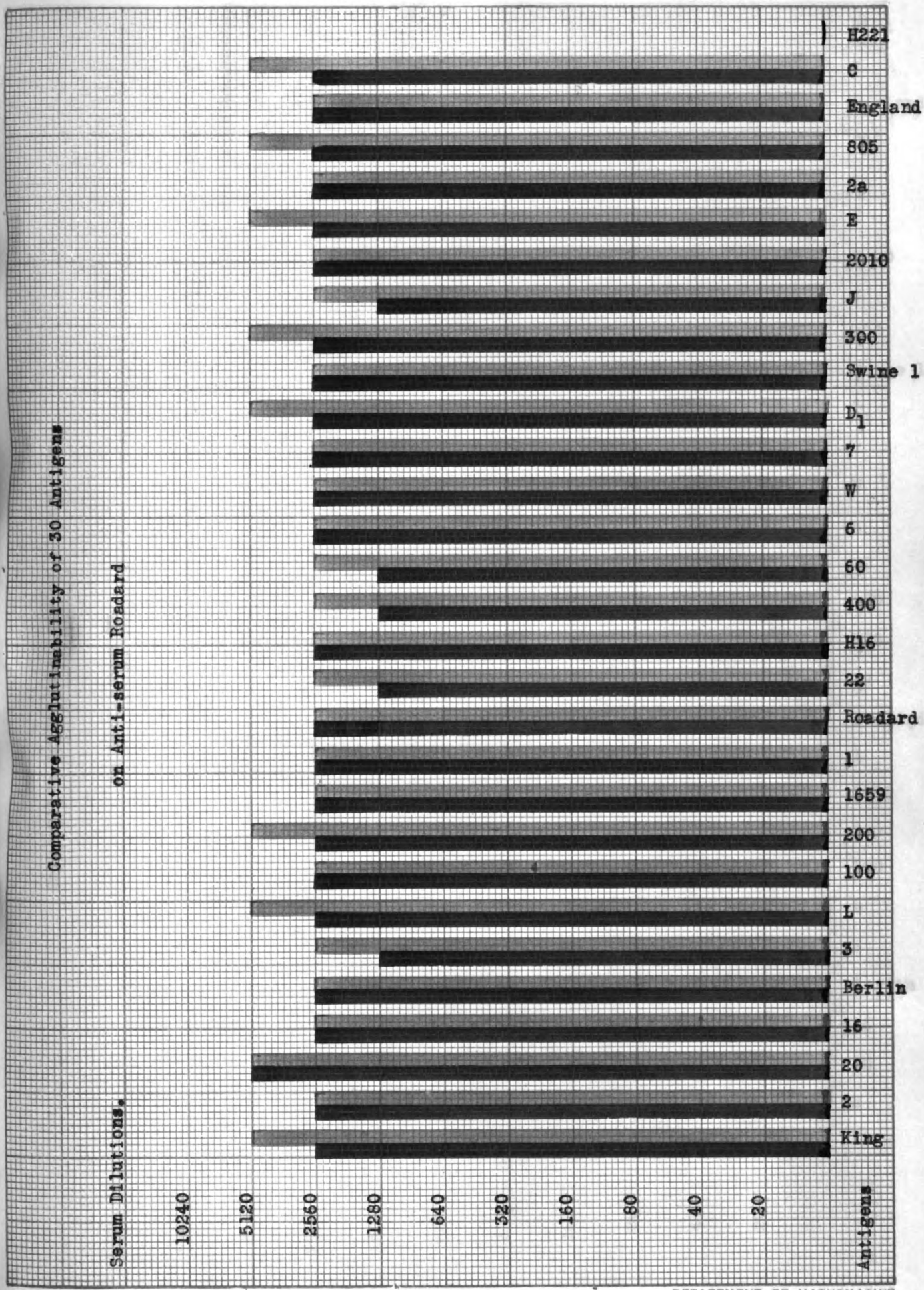
400
H16
22
Rozdard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum Roadard
Serum Dilutions.
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40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
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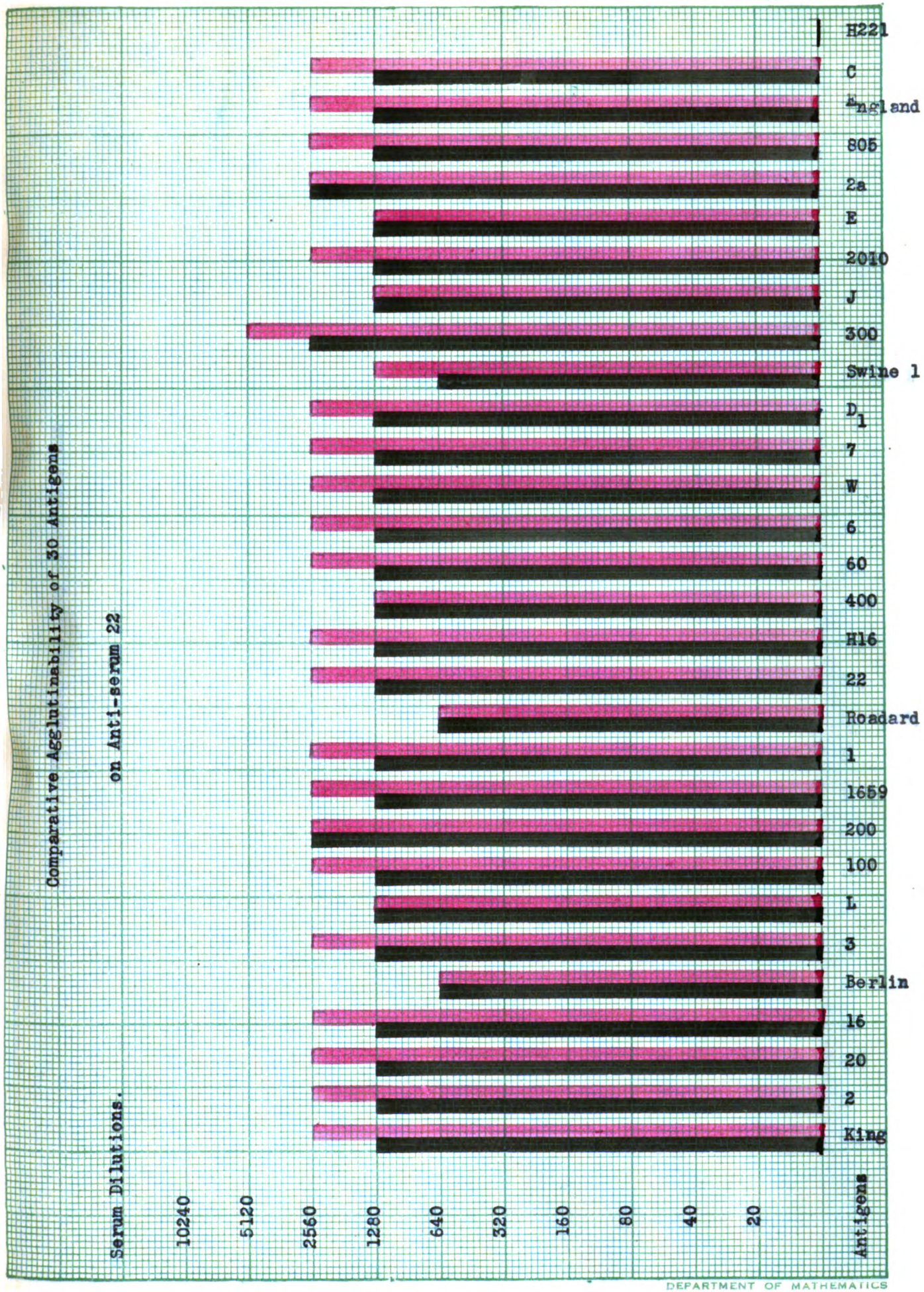
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum 22
Serum Dilutions.
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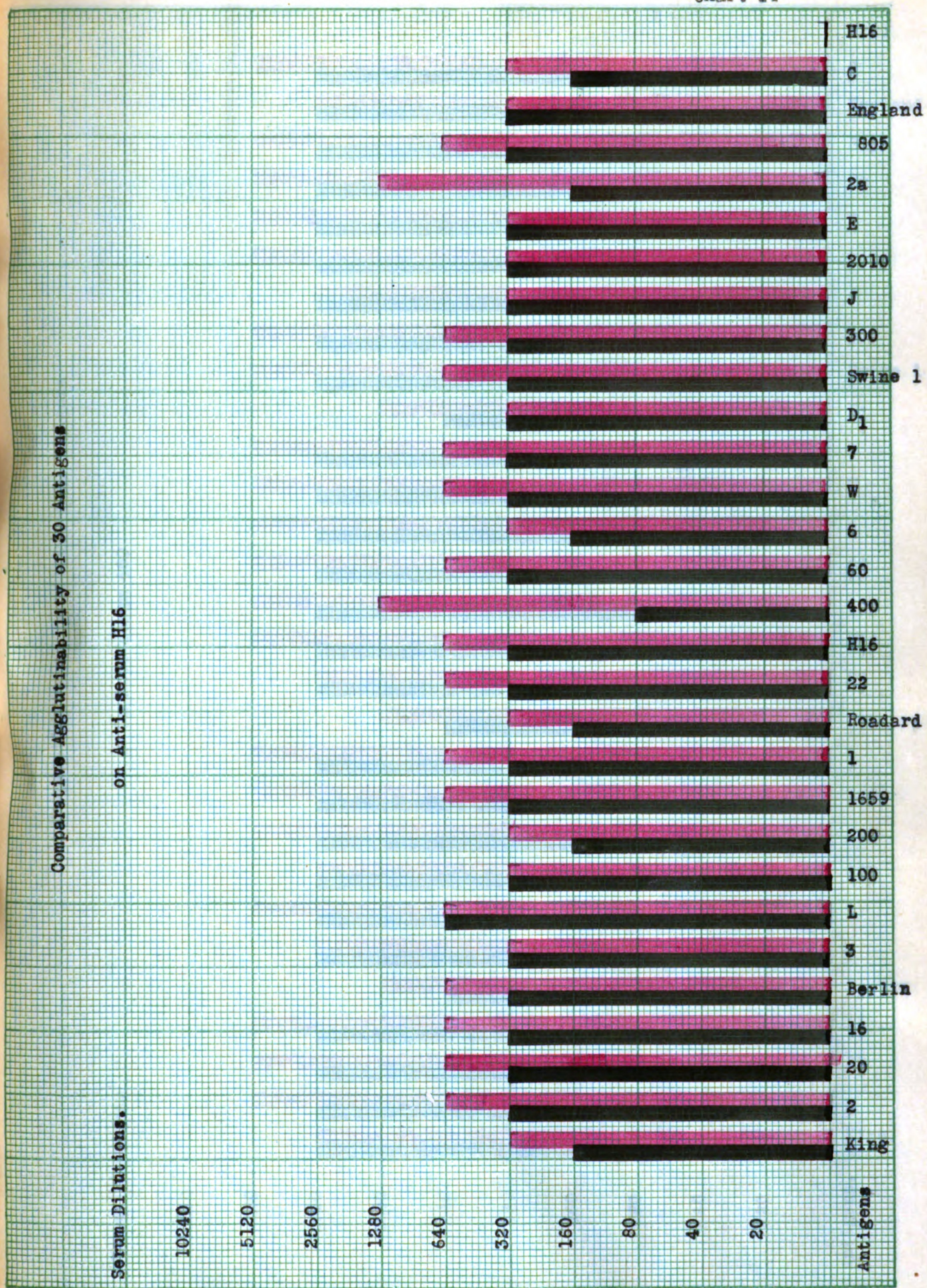
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum H16
Serum Dilutions.
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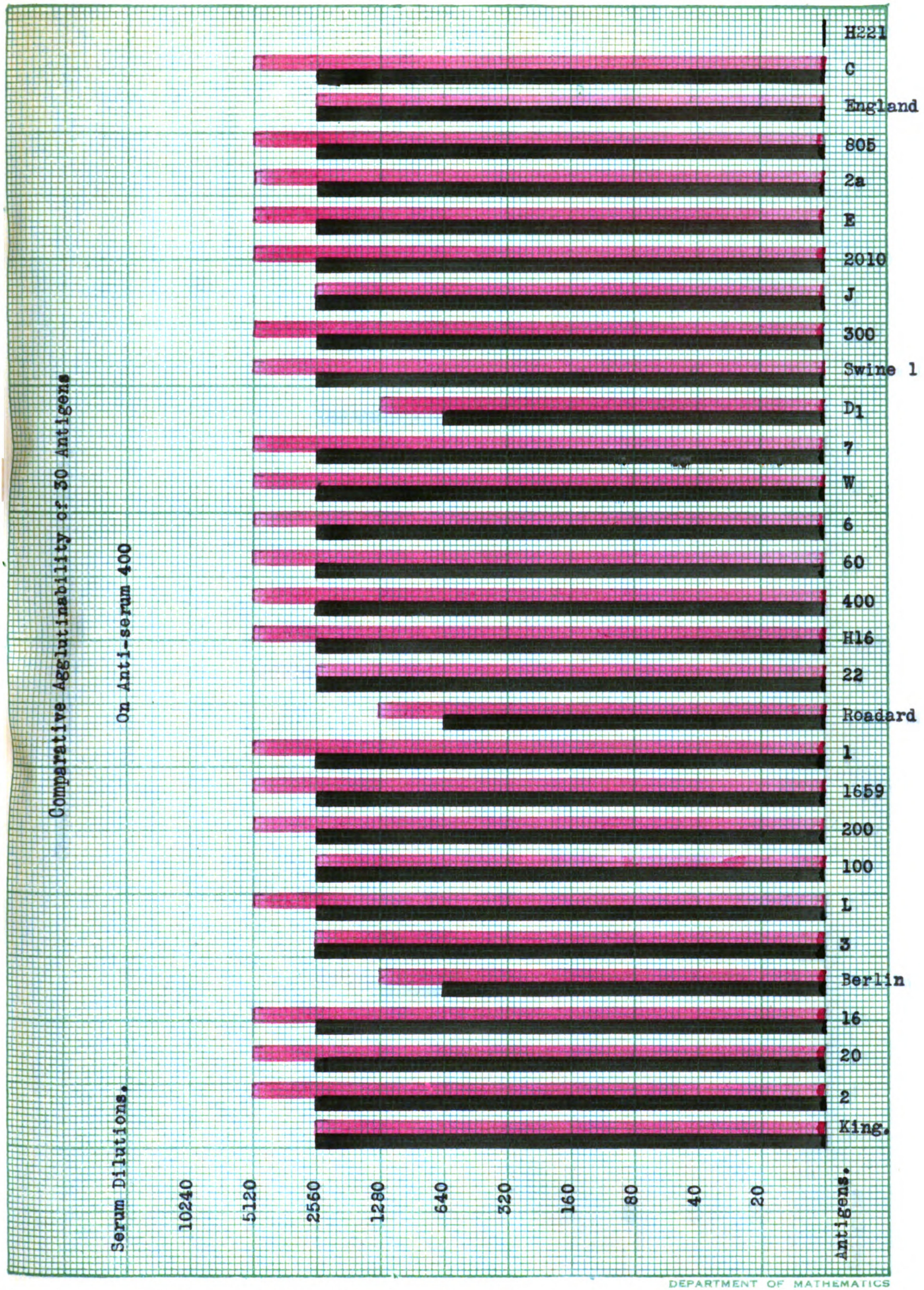
2560
1280
640
320
160
80
40
20
Antigens
H16
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-serum 400
Serum Dilutions.
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5120
2560
1280
640
320
160
80
40
20
Antigens.
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
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W
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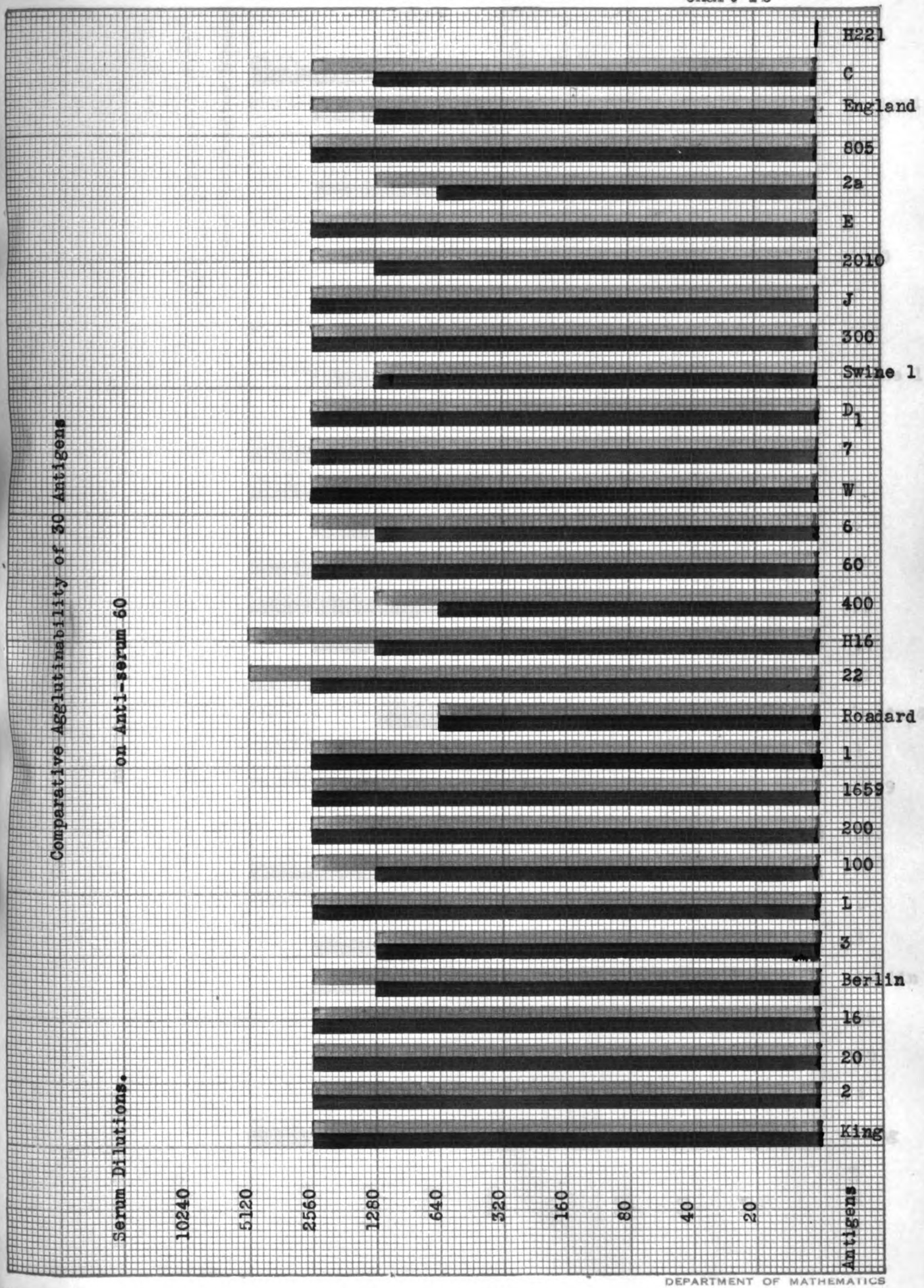
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King.


Comparative Agglutinability of 30 Antigens
on Anti-serum 60
Serum Dilutions.
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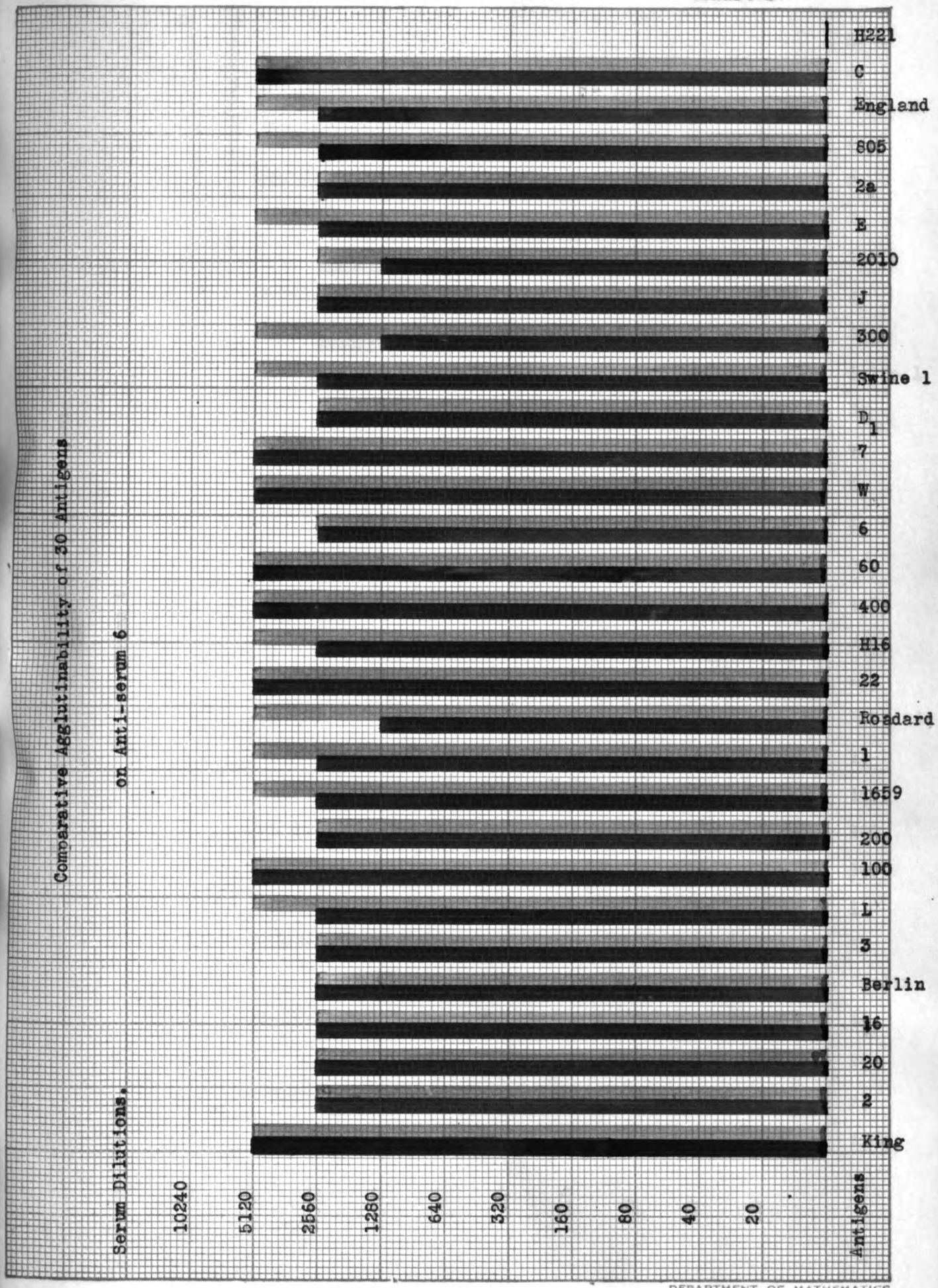
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum 6
Serum Dilutions.
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5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
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400
H16
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Roadard
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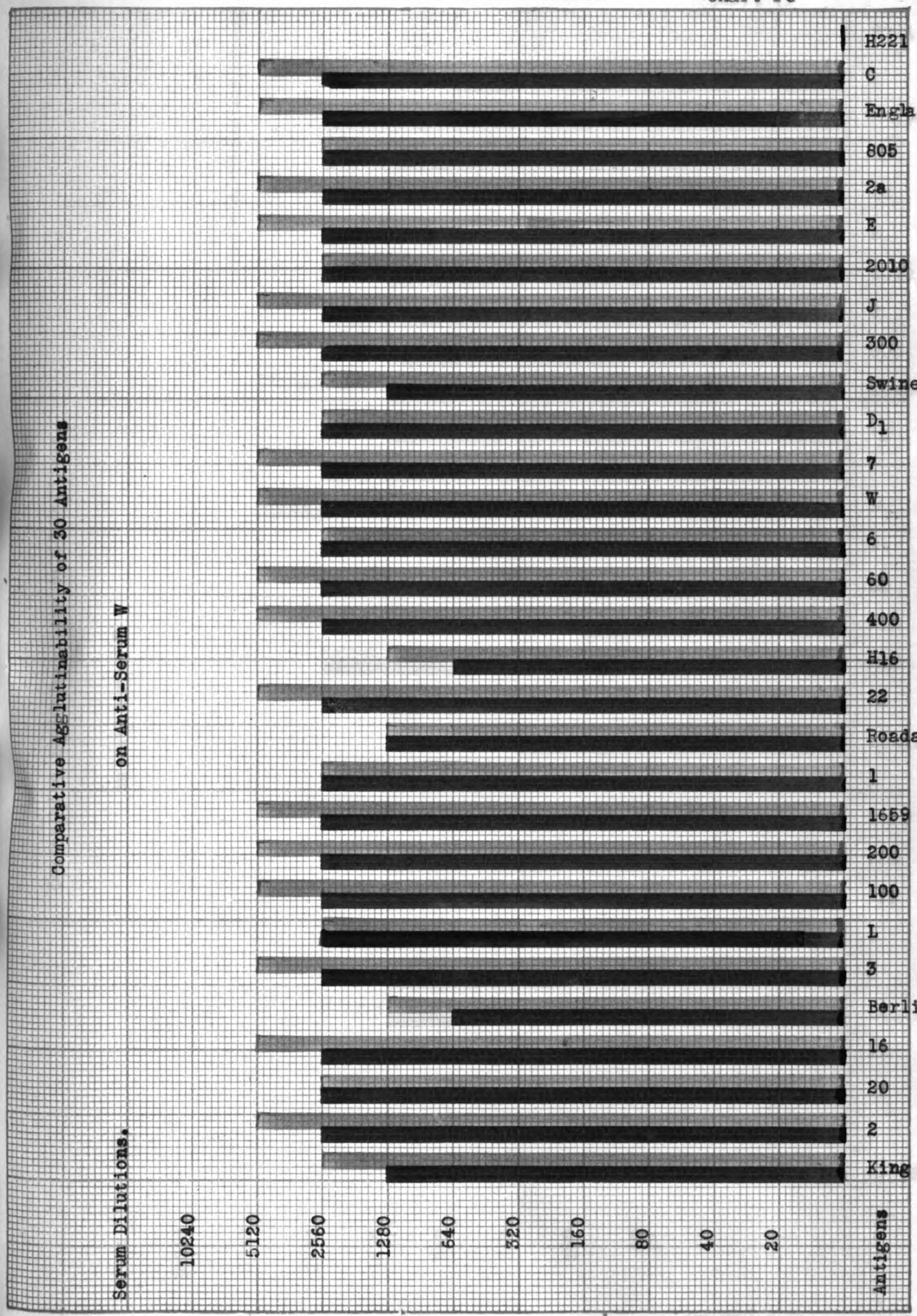
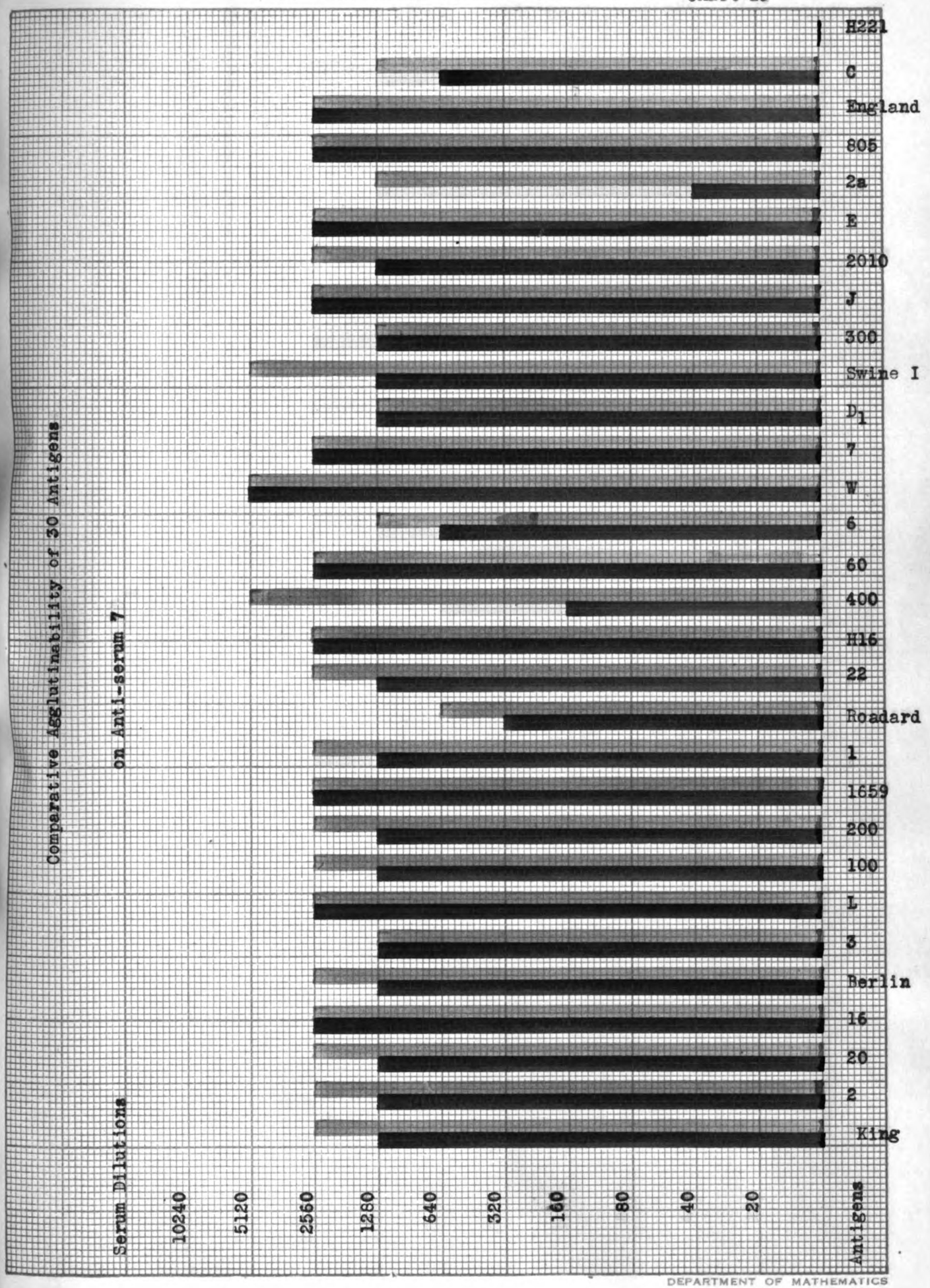
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-Serum W
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King

Comparative Anatomy of 30 Antigens


Comparative Agglutinability of 30 Antigens
on Anti-serum 7
Serum Dilutions
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
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H16
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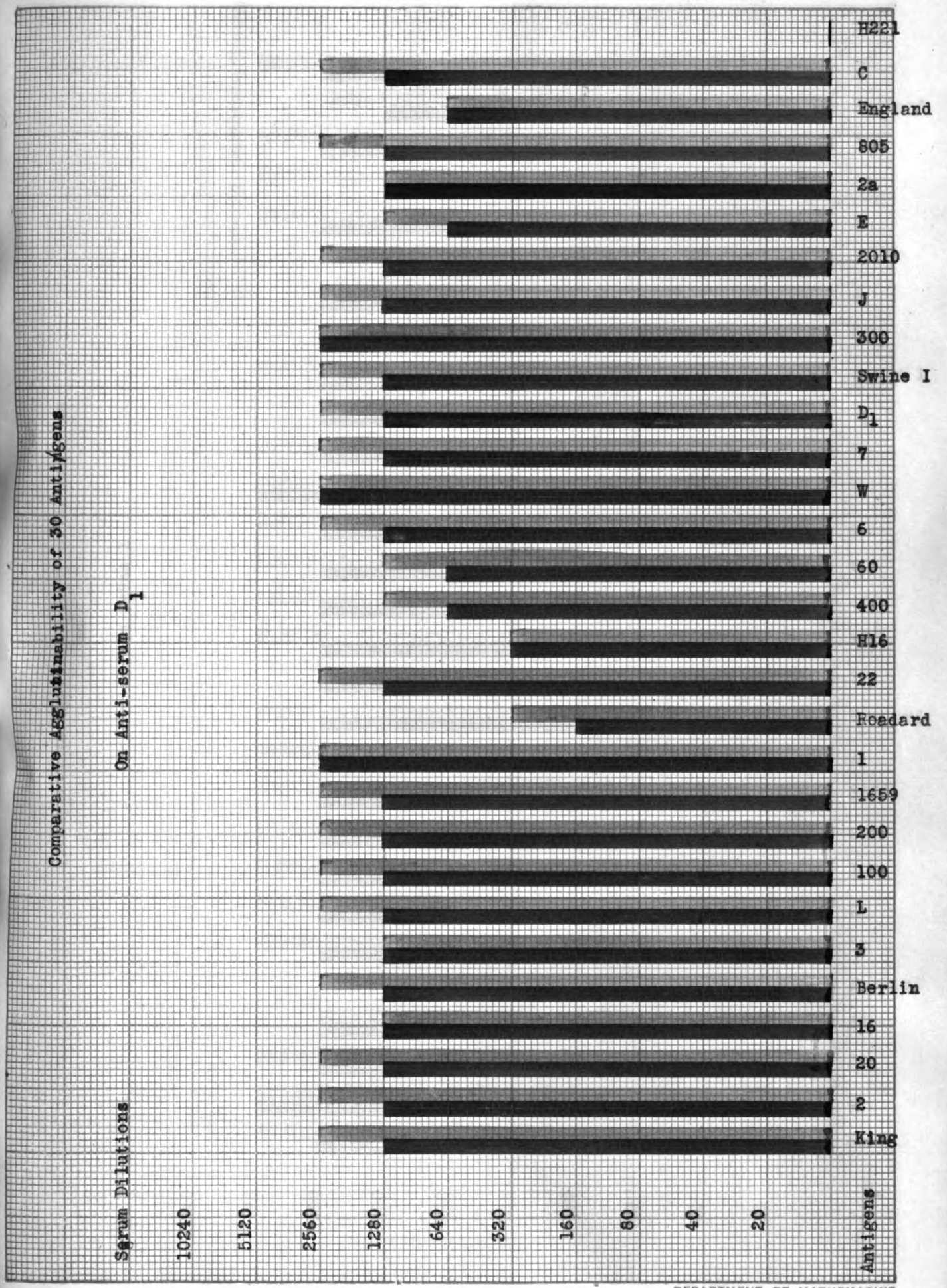
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-serum D1
Serum Dilutions
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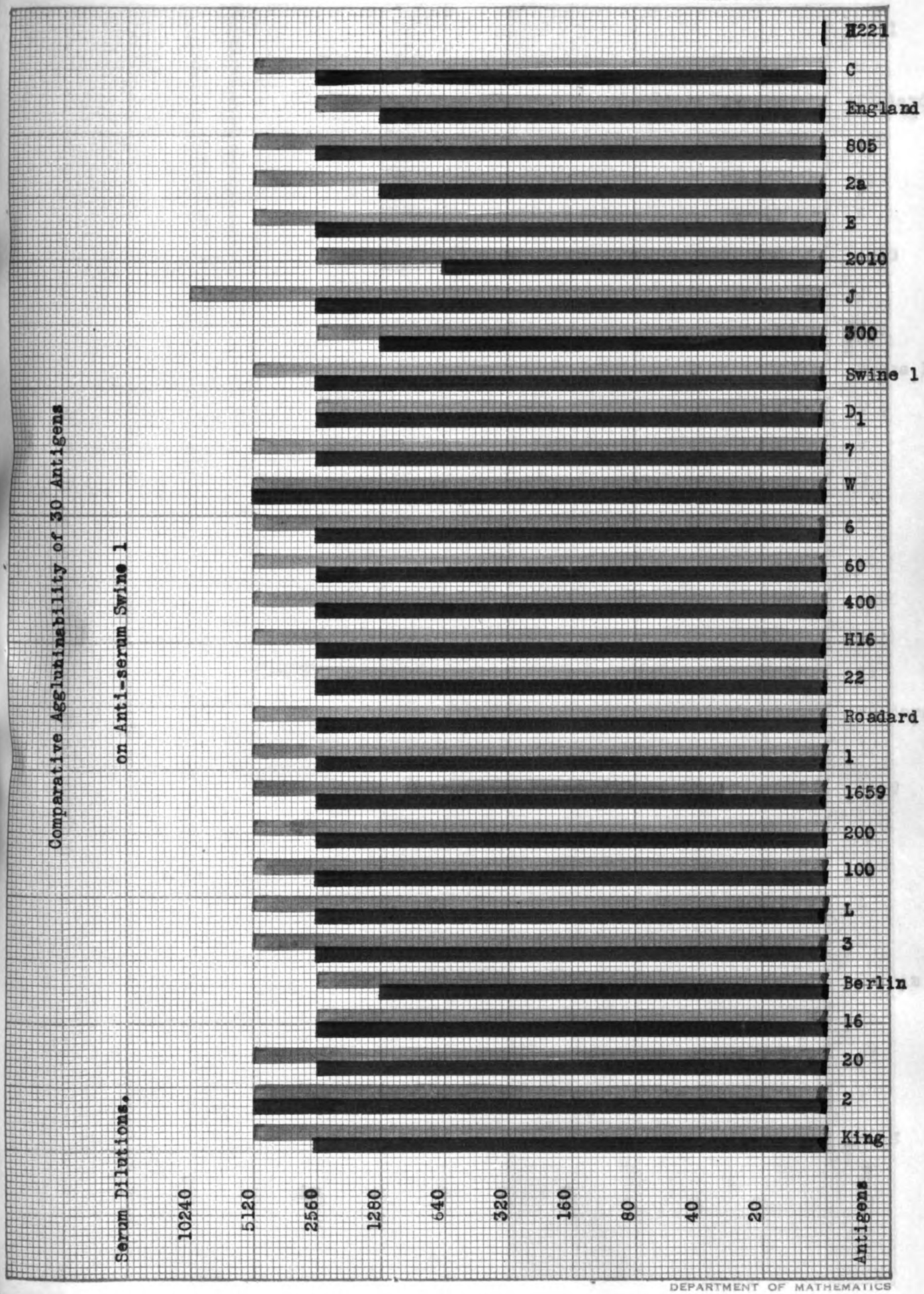
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum Swine 1
Serum Dilutions.
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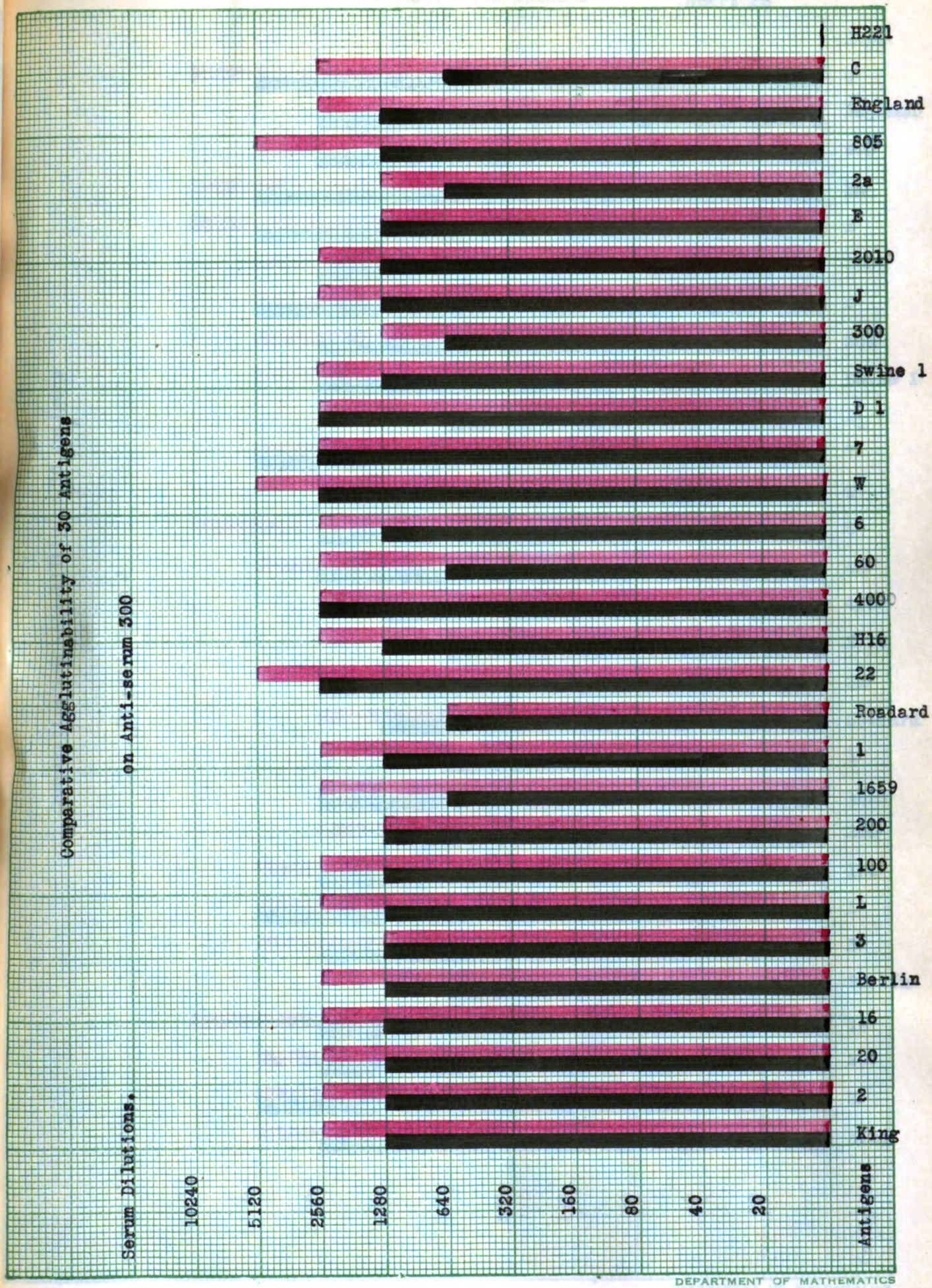
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King

Comparative Agglutinability of Bφ Antigens


Comparative Agglutinability of 30 Antigens
on Anti-serum 300
Serum Dilutions.
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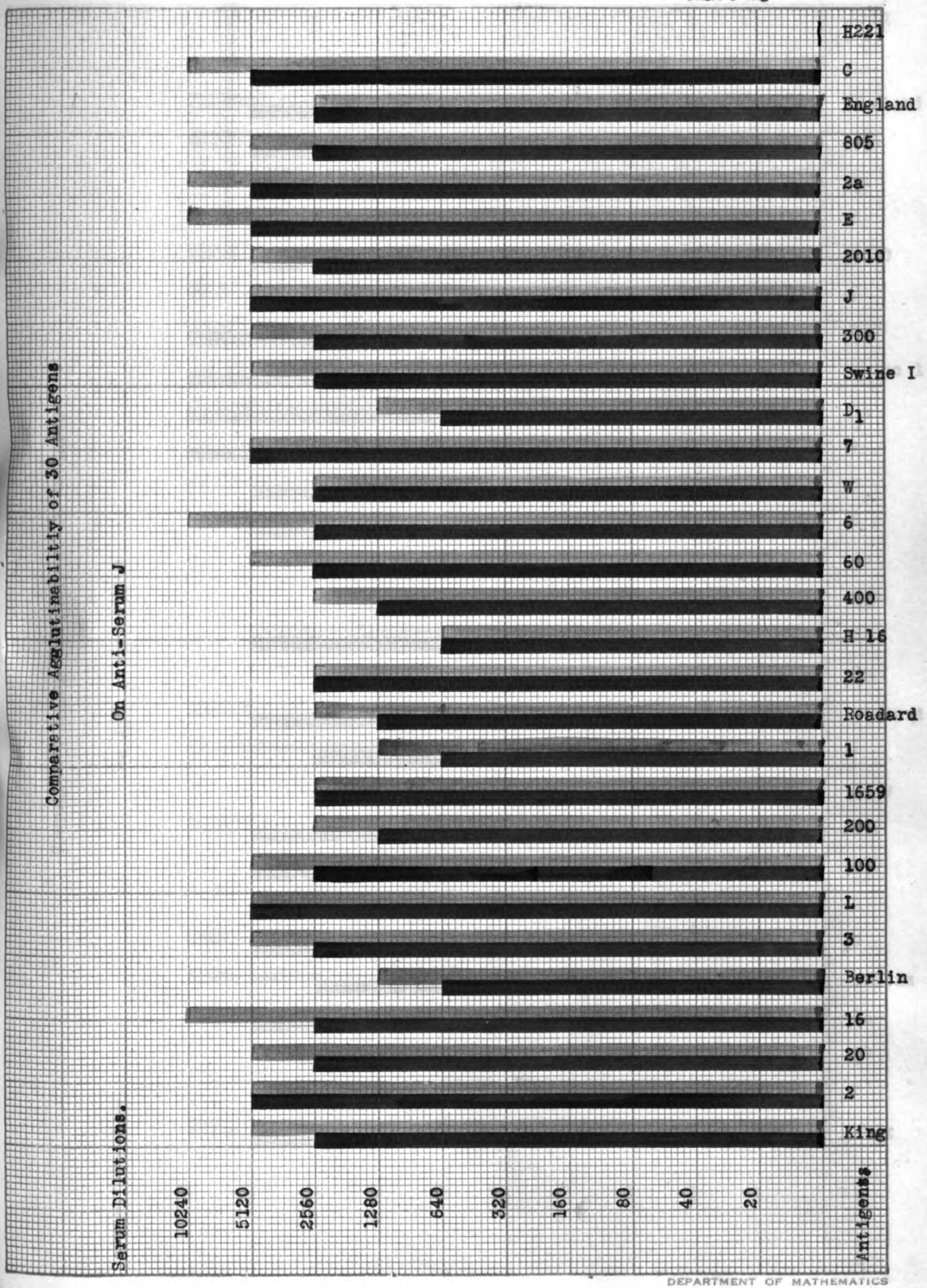
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D 1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-Serum J
Serum Dilutions.
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2560
1280
640
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160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
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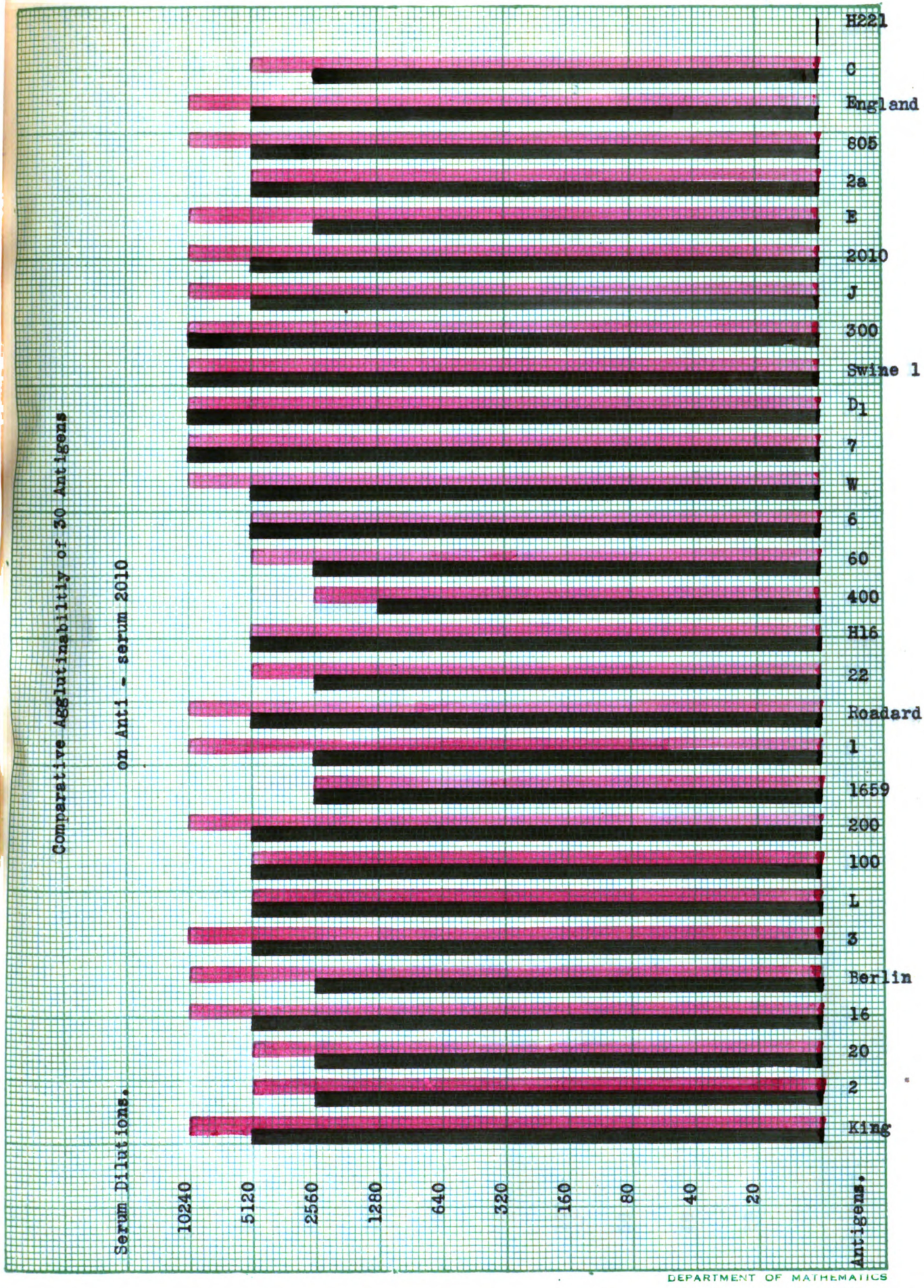
H 16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti - serum 2010
Serum Dilutions.
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5120
2560
1280
640
320
160
80
40
20
Antigens.
H221
0
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
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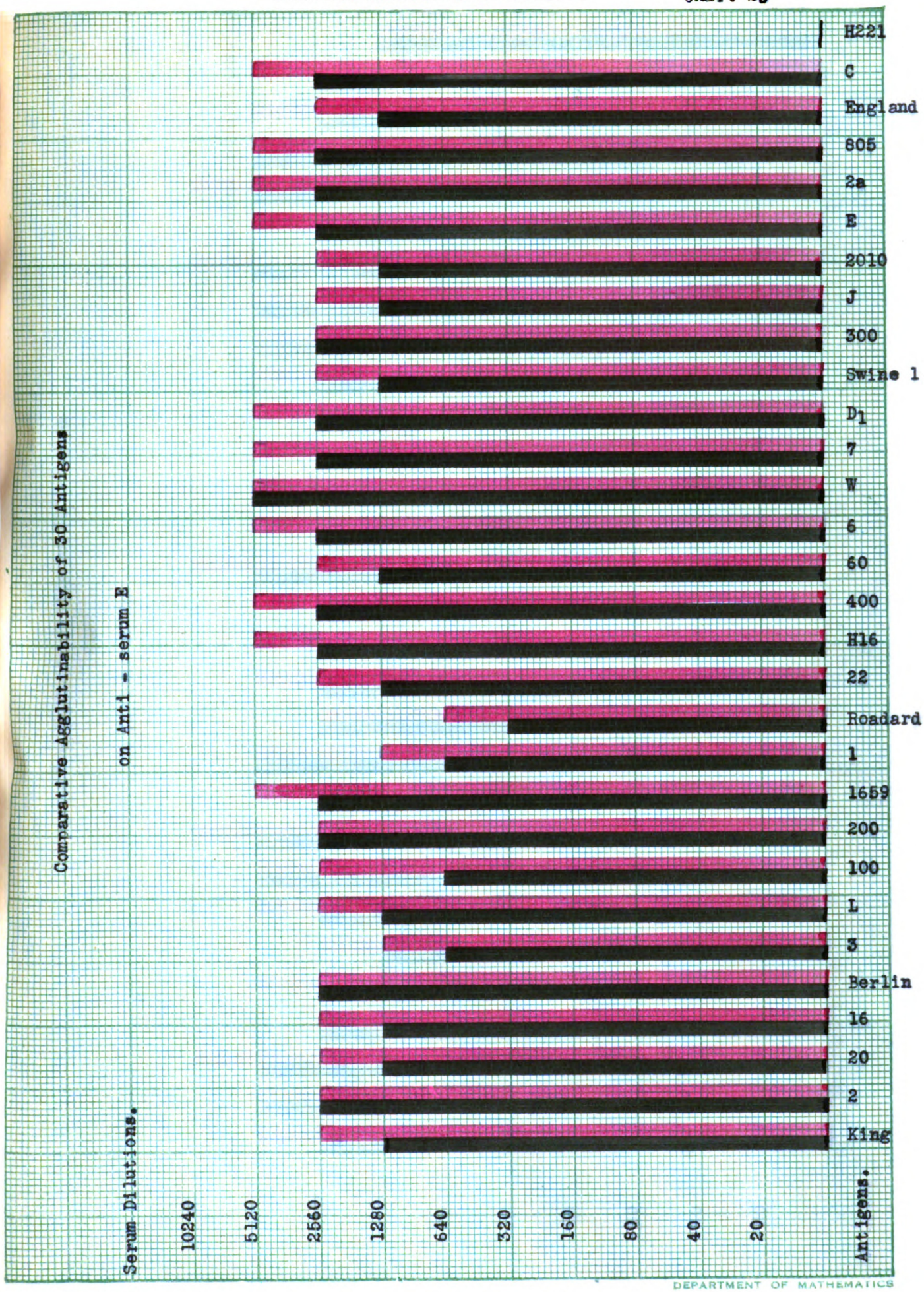
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King

Comparative Agglutinability of 50 Antigens


Comparative Agglutinability of 30 Antigens
on Anti - serum E
Serum Dilutions.
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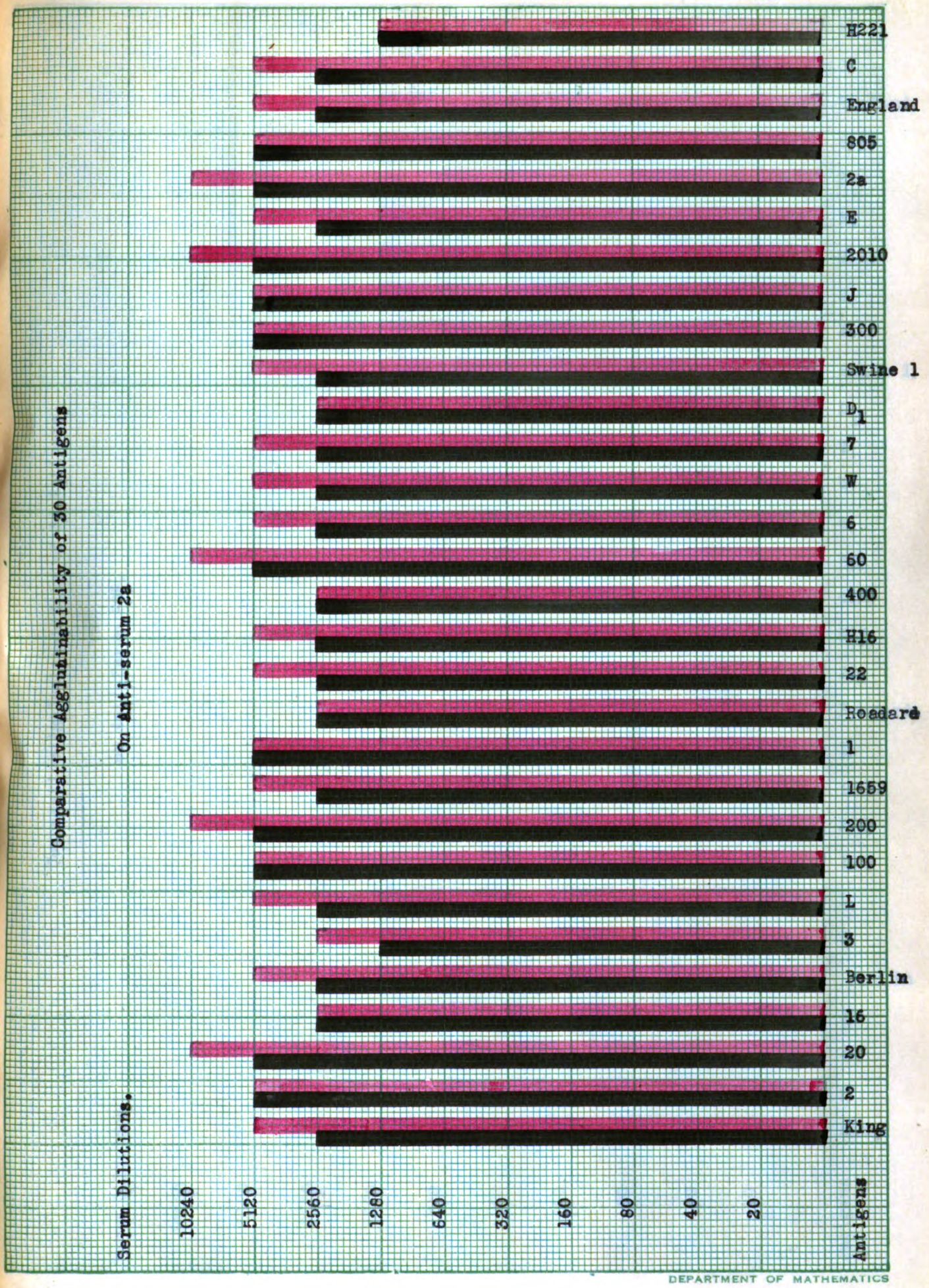
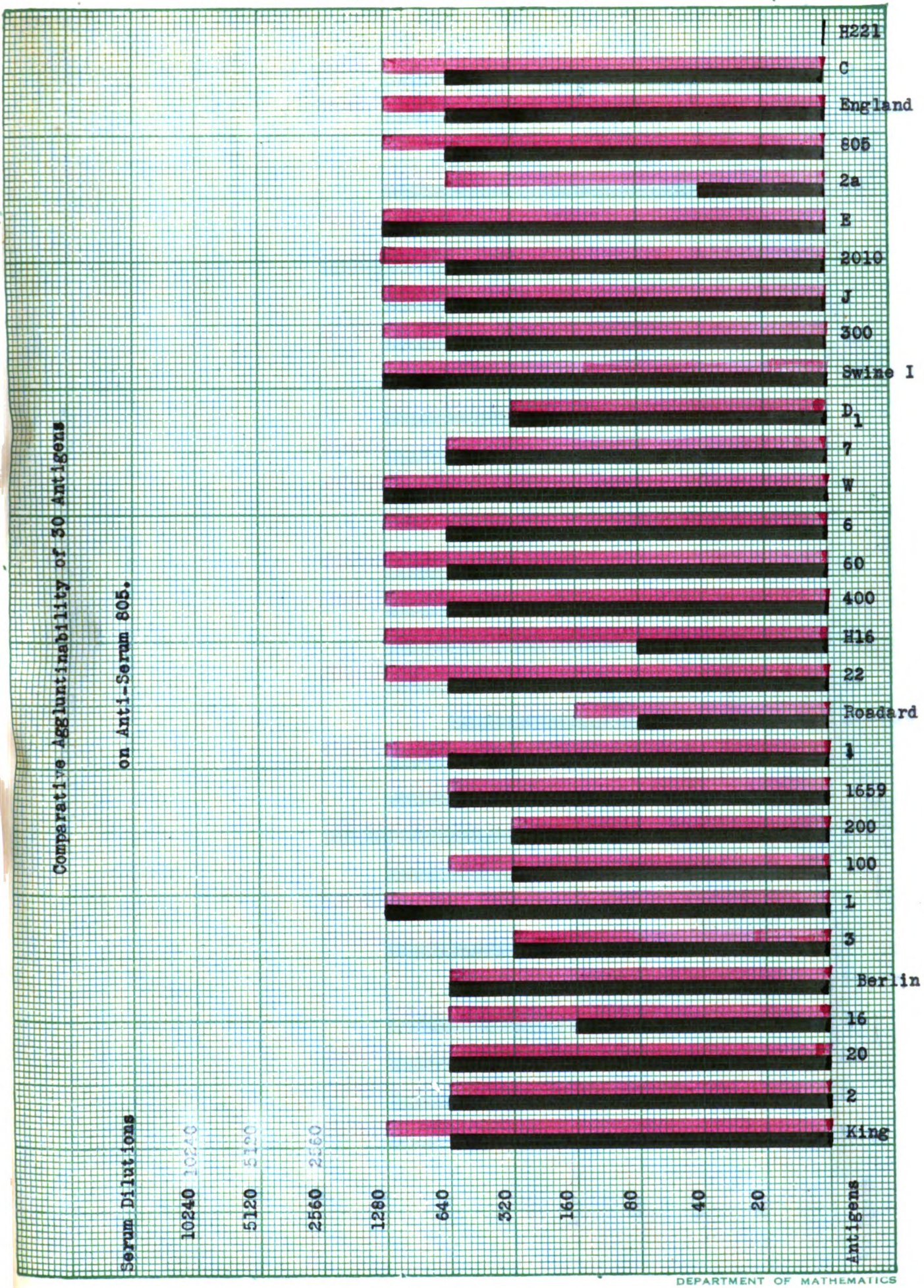
5120
2560
1280
640
320
160
80
40
20
Antigens.
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King

Comparative Agglutinability of 30 Antigens


Comparative Agglutinability of 30 Antigens
On Anti-serum 2a
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadaré
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-Serum 805.
Serum Dilutions
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine I
D1
7
W
6
60
400
H16
22
Rosdard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
On Anti-serum England
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
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60
400
H16
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Roadard
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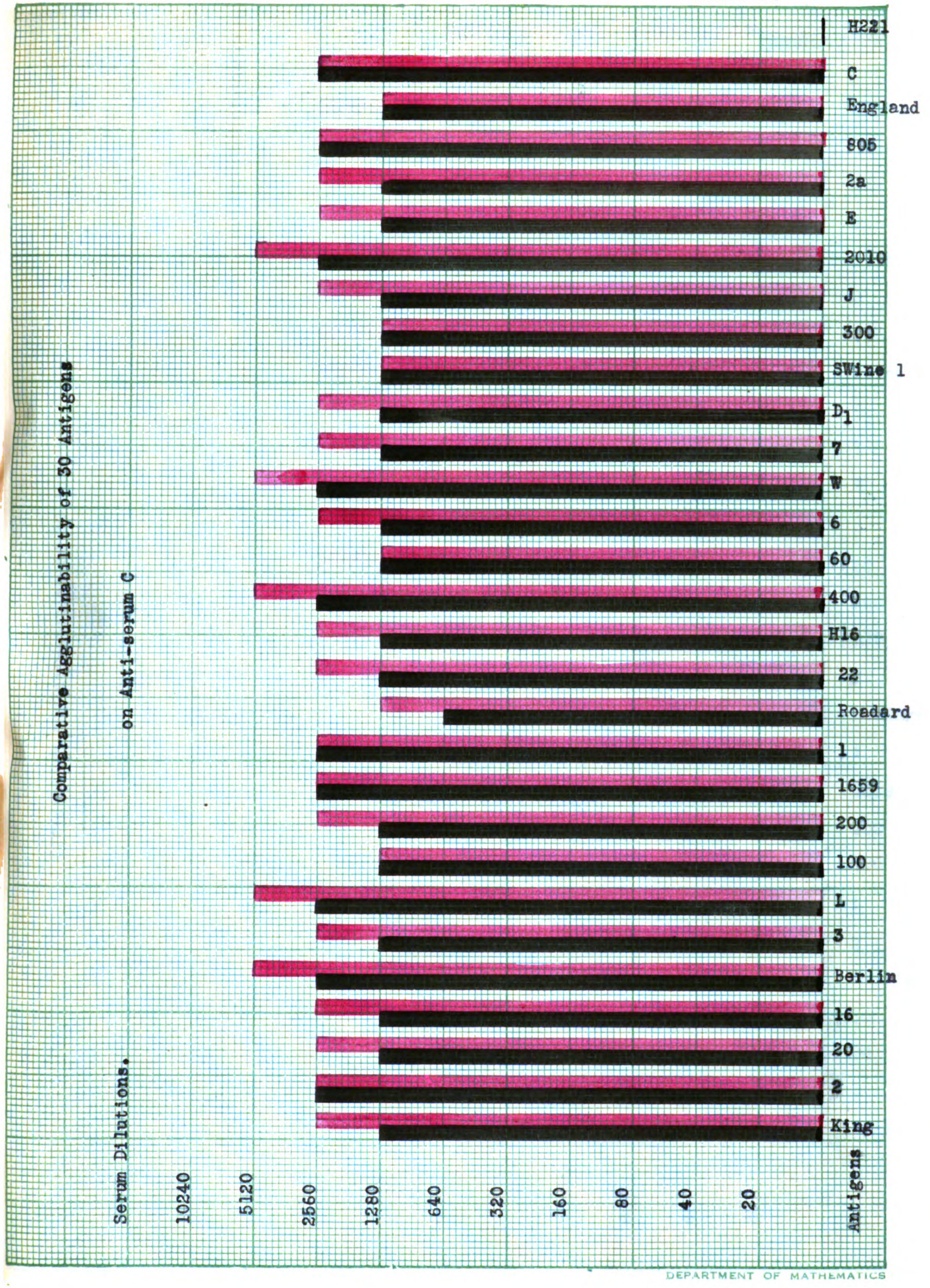
1659
200
100
L
3
Berlin
16
20
2
King

Chart 29


Comparative Agglutinability of 30 Antigens
on Anti-serum C
Serum Dilutions.
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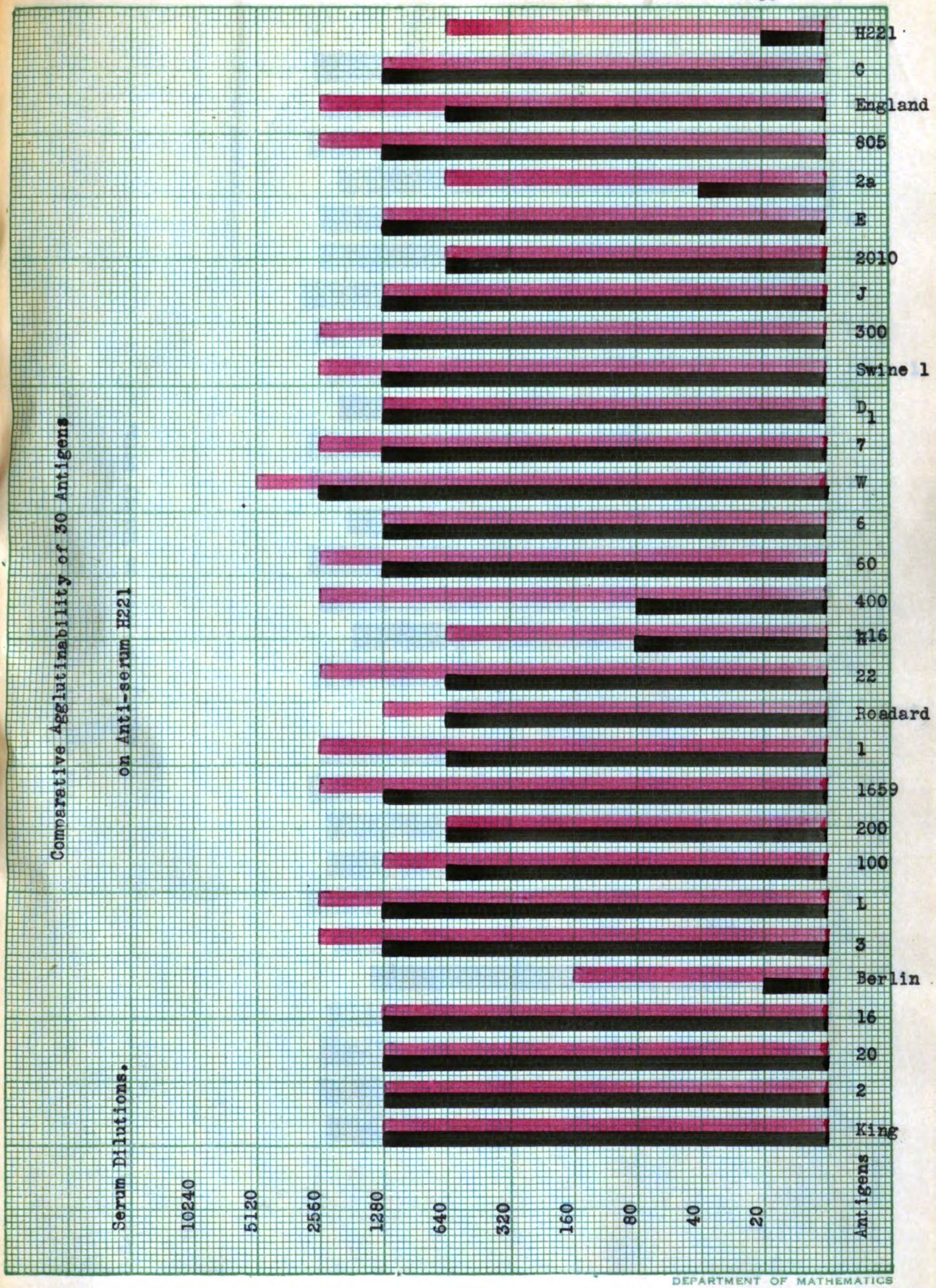
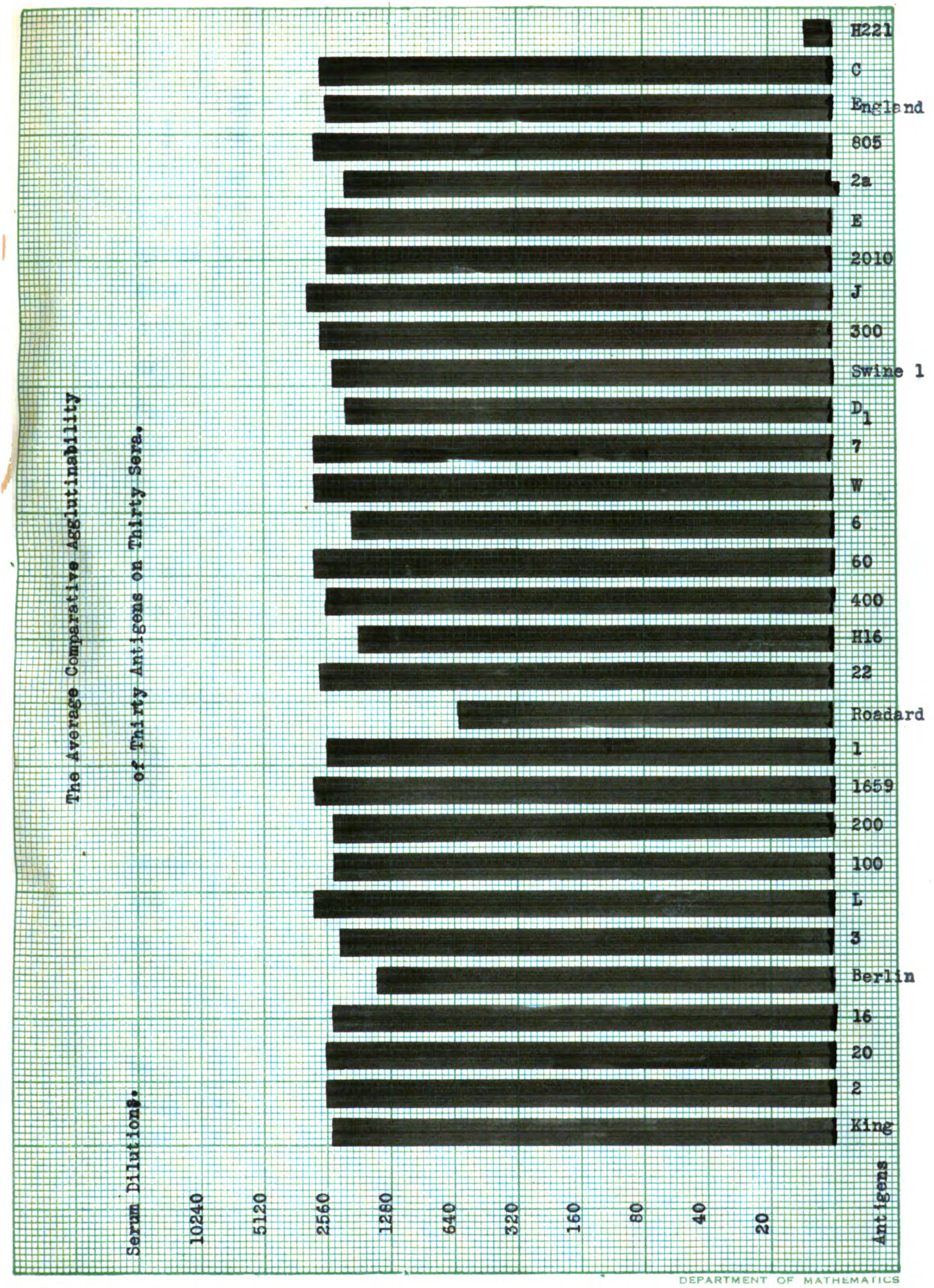
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
SWine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King


Comparative Agglutinability of 30 Antigens
on Anti-serum H221
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Readard
1
1659
200
100
L
3
Berlin
16
20
2
King


The Average Comparative Agglutinability
of Thirty Antigens on Thirty Sera.
Serum Dilutions.
10240
5120
2560
1280
640
320
160
80
40
20
Antigens
H221
C
England
805
2a
E
2010
J
300
Swine 1
D1
7
W
6
60
400
H16
22
Roadard
1
1659
200
100
L
3
Berlin
16
20
2
King

Antigens.	Ab Se 40
100	-
Berlin	-
2010	-
C	-
22	-
D_1	-
H16	-
2	-
300	+
King	-
805	-
6	-
England	-
J	-
L	-
7	-
Roadard	-
400	-
60	-
3	-
E	-
2a	-
Swine 1	P
H221	P
1	-
W	-

Table 3.

Results of Agglutination after the Absorption of Serum 400 with Antigen 2a

Antigen	Serum Dilution 1 to 40	80	160	320	640
100	-	-	-	-	-
Berlin	-	-	-	-	-
2010	-	-	-	-	-
C	-	-	-	-	-
22	-	-	-	-	-
D_1	-	-	-	-	-
M16	-	-	-	-	-
2	-	-	-	-	-
300	-	-	-	-	-
King	-	-	-	-	-
805	-	-	-	-	-
6	-	-	-	-	-
England	-	-	-	-	-
J	-	-	-	-	-
L	-	-	-	-	-
7	-	-	-	-	-
Roadard	-	-	-	-	-
400	-	-	-	-	-
60	-	-	-	-	-
3	-	-	-	-	-
E	-	-	-	-	-
2a	-	-	-	-	-
Swine 1	-	-	-	-	-
M221	-	-	-	-	-
1	-	-	-	-	-

pH of Antigen			
4.0			
4.4			
4.8	-	-	-
5.2	-	-	-
5.6	-	-	-
6.0	-	-	-
6.4	-	-	-
6.8	-	-	-
7.2	-	-	-
7.6	-	-	-
8.0	-	-	-
8.4	-	-	-
8.8	-	-	-

Discussion.

From the data obtained in cross agglutination it may be seen that no two antigens were agglutinated to the same degree by each of the thirty sera. However, there were many antigens which were agglutinated to the same degree by a single heterologous serum. Chart number (8) shows that antigen 100 produced agglutinins to a markedly low degree for antigens 2a, 400, and H16. This occurrence is difficult to explain as no other serum agglutinated these three antigens to such a low degree. In many cases the agglutinins produced by an antigen gave a much higher titre for heterologous antigens than for the homologous antigen. The charts show that strain H221 differed markedly in its agglutinability from the other strains, or the agglutinins produced by the other strains with the exception of # 2a were of a different type than those which are necessary to cause a clumping of this antigen. If H221 is to be considered alone, some reason must be given for such an individual inagglutinability. Krumwiede, Cooper, and Provost (31) report the possibility of existence of degraded variants. They describe such strains as having lost their agglutinability and a marked loss in antigenic power. In this case H221 seems to have a very low agglutinability but possesses an average antigenic power. Therefore, one of its properties function, while another has degraded or has been lost almost entirely. Krumwiede (31) and his co-workers also state that a degraded variant may stimulate the production of agglutinins which it is relatively unable to bind. One may notice that H221 antigen is agglutinated in relatively low dilutions by its homologous serum, as compared to heterologous strains.

All of the other antigens stimulated the production of agglutinins of a fairly high titre for both the homologous and heterologous. It is possible to conclude from this reasoning that strain H221 is a degraded variant.

The fact that serum 2a agglutinated antigen H221, however, cannot be explained by the above reasoning. But let us refer to the agglutinin absorption test for a little information. When serum H221 was absorbed by its homologous strain it removed the agglutinins for & 2a also. When serum 2a was absorbed by its homologous strain it removed the agglutinins for H221 as well. Therefore, we may be led to believe that these two strains are closely related.

In considering the final results of cross agglutination it is readily apparent that no group separation of the thirty strains used is possible, except, possibly in the case of strain H221 which might be placed in a distinct group or the term degraded variant applied according to Krumwiede, Cooper, and Provost (31).

It seems that the agglutinability of a given strain is of considerable importance, especially from the standpoint of accurate diagnosis and the obtaining of comparable results by different laboratories engaged in the performance of the agglutination test. The charts reveal that antigens prepared from different strains are not agglutinable to the same degree by a given serum. This finding in all probability explains the great degree of variation of the agglutination titre obtained by different laboratories on the same serum.

In the agglutinin absorption test, after absorbing the sera with their homologous strain, agglutinins remained to a low degree for heterologous strains in every instance but one. Strain 400 removed from its homologous serum its homologous agglutinins and

those for 25 heterologous strains. In this case strain 400 was able to remove all agglutinins, the production of which was due to the stimulus of this strain. Krumwiede (31) and his co-workers believe that such a statement can safely be made, but they report two exceptions to this. First, normal agglutinins may be present; second, agglutinins whose production was stimulated by degraded variants may be present. They state that all of the latter agglutinins are not always absorbed by the degraded variants which stimulated their production. We will consider these exceptions under those cases where agglutinins for heterologous strains were present after absorption.

When serum 400 was absorbed by heterologous strain 2a both homologous and heterologous agglutinins were removed. This tends to show that strains 400 and 2a may be very closely related.

The seven sera which contained heterologous agglutinins, after homologous agglutinins absorption, present a different problem. We might explain their presence by calling them normal agglutinins, but the sera of the rabbits were tested before injection for agglutinins, and all gave negative results. The presence of heterologous agglutinins might be explained by refering to the strains which stimulated their production, as degraded variants. Is it logical to say that out of eight strains, only one could be classed as being normal, and the remaining seven as degraded variants? The presence of heterologous agglutinins in the absorbed serum, might be explained by calling them non-specific agglutinins. It seems possible for a strain to stimulate the production of two kinds of agglutinins, namely specific and non-specific. The specific agglutinins would be absorbed by the homologous strain only, while the non-specific agglutinins would remain in the serum. If a homologous strain removed its homologous agglutinins and also removed others which were thought to be heterologous, all would have to be

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classed as specific. This occurred when serum Roadard was absorbed by its homologous strain, for the agglutinins for Berlin, King, and 2a were also removed. Thus the remaining agglutinins were non-specific, and also the homologous strain was unable to remove them. This also occurred in the other six sera. Therefore, it seems possible to assume that a strain may stimulate the production of both specific and non-specific agglutinins. Variations have been noticed in the agglutination tests of various laboratories on the same sera. Investigators are desirous of eliminating all possible variations in this test with the ultimate view of standardization. It was thought that the pH of the antigen might be one of the causes ofvariation in agglutinability, so a series of tests were carried out using antigens of different pH. The only variations noticed in these tests were recorded in antigens with a pH of 4.0, 4.4, and 4.8. It seemed that in these antigens, the concentration of the acid was so strong, that it had the property of causing the albumin of the serum to be precipitated to the bottom of the tubes in a flocculant mass. This interfered with the reading of the tests, so it was thought advisable to use an antigen whose pH was somewhat higher than 4.8. The antigens used in the cross agglutination and agglutinin absorption work had a pH of 6.8 and all of the reactions were easily read and no precipitation was noticed. No differences were noted in agglutinating power in antigens from pH 5.2 to pH 8.8. The alkaline antigens did not appear to interfere with agglutination. So, if an average is taken between these two extremes it should give an antigen of a pH which would give satifactory results. This numberical average is pH 7.0. The literature shows that agglutination test is more efficient if the antigen is slightly acid. So, if the pH of the antigen to be used in the test is 6.8, it is believed that the results of the test would be much more constant.

Conclusions.

Cross agglutination does not serve to group the thirty strains of Bacterium abortus used in this study, although one strain appeared to be of a different group.

The agglutinin absorption test as used shows no specific differences between the twenty-six strains of Bacterium abortus used. This test, however, has possibilities and should be given further study.

The hydrogen ion concentration of an antigin does not effect its agglutinability unless the antigin has a pH of 4.8 or below.

The writer wishes to acknowledge his indebtedness to Mr I. Forrest Huddleson, Mr. W.L.Mallman, and Dr. Ward Giltner for the assistance and helpful suggestions received during the course of these studies.

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