3 tissues listed above. Except for the first week or two following pancreatectomy, during which time their appetites were somewhat poor, both dogs have had voracious appetites throughout their stay in the laboratory. Both dogs are still alive and cannot be distinguished from normal dogs in appearance or reactions.

8313 C

Measurement of Reagin in Non-Syphilitic Sera.*

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(Introduced by M. L. Tainter.)

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The reporting of serological tests for syphilis as positive or negative implies a qualitative difference between the bloods of syphilitic and non-syphilitic individuals. This concept assumes the presence in the serum of syphilitic patients of a substance designated as "reagin", which is lacking in patients without syphilis. In syphilitic patients, treated or untreated, it is highly probable that small amounts of reagin persist even though the serum is negative to the usual tests. As yet there is no evidence that reagin may not also be present in non-syphilitic sera.

If reagin is to be demonstrated in non-syphilitic sera, it is necessary to use a test so sensitive as to be invariably positive, to concentrate the serum, or to increase the reagin content to a point where it can be detected by the usual tests. We have chosen the last method, adapted from the work of Schreus and Foerster[1] who studied the Wassermann reaction in syphilitic patients treated to seronegativity. By adding to the sera of these patients subthreshold amounts of positive sera, these workers were able to obtain positive reactions. In place of the Wassermann we have substituted the Kline test because of its simplicity.

Suspensions are prepared from standard Kline antigen in the usual way. A stock reagin solution is prepared from positive serum after inactivation for 15 minutes at 56°C. Double precipitation is

* This work has been aided by a grant from the Rockefeller Fluid Research Fund.

[1] Schreus, H., and Foerster, R., *Z. f. Immunitäforsch. u. Exp. Therap.*, 1934, **82**, 53.

carried out in half saturated ammonium sulphate as described by Schreus and Foerster. The second precipitate is dissolved in a minimal amount of normal saline. The reagin content of this stock reagin solution decreases less rapidly than that of untreated serum according to these workers. This solution is now diluted 10, 20, 30, 40, and 50 times with saline and the Kline test[2] is performed with these dilutions to determine the approximate titre. Further tests are done on dilutions made in unit steps within the range indicated by the first titration and the exact titre is thus obtained.

Reagin is added to the serum to be titrated as indicated in Table I.

TABLE I.*

Test No.	Serum cc.	Reagin cc.	Saline cc.
1	.05	—	—
2	.04	.001	.009
3	.04	.002	.008
4	.04	.003	.007
5	.04	.004	.006
6	.04	.005	.005
7	.04	.006	.004
8	.04	.007	.003
9	.04	.008	.002
10	.04	.009	.001
11	.04	.01	—
12	.039	.011	—

*These quantities are too small for accurate measurement; the solutions in each test are mixed in the same proportions but in larger quantities and .05 cc. of this mixture is taken for the test.

The quantities indicated in Table I are selected to bring the total volume of fluid in each test to .05 cc. as required in the Kline test. When serum is replaced by normal saline, all tests up to No. 11 should be negative, and 11 and 12 positive. This result will be obtained if a dilution of stock reagen to 1/5 of its titre is used. Thus if the stock reagin has a titre of 1:36, the dilution to be used is 1:36/5 or 1:7.2. Such a dilution is prepared and the series of tests shown in Table I is set up using saline instead of serum to verify the original titration. The tests are then repeated with serum inactivated for 15 minutes at 56°C. If no reagin is present in the serum, there will be no shift in the end point and test No. 11 will again be the first to be positive. If, however, reagin is present, this point will be shifted and the degree of this shift will indicate the amount of reagin present.

There is no standard unit of reagin. For the purpose of this study we have taken as our unit of reagin the least amount that can

[2] Kline, B. S., and Young, A. M., *J. Lab. and Clin. Med.*, 1927, **12**, 477.

be detected by this technic. The end point is the first test showing agglutination. The reading of this end point is made possible by having a series of negatives on one side and a series of increasing positives on the other for comparison. Sharp end points are obtained only when freshly prepared cholesterin solutions are used in preparing the Kline antigen suspension. All tests have been read independently by the 3 of us and the errors in the readings have never been more than one-tenth of a unit.

If such a series of tests is done with an unknown serum we may find that the first positive test is No. 7 instead of No. 11 as in the saline control series. This indicates the presence in the serum of 0.4 units of reagin. Since the serum has been diluted by the addition of saline and reagin, this figure must be multiplied by 5/4 to obtain the actual reagin content. This would give us in this example $0.4 \times 5/4 = 0.5$ units.

In order that our unit of reagin will be constant the sensitivity of the test must remain fixed. The fixed sensitivity of the test was verified by the preparation of 9 separate pairs of antigen suspensions. Five of these pairs gave identical results when used on the same serum or reagin solution, and 4 disagreed by only 0.1 units. Fresh suspensions and suspensions 24 hours old showed the same agreement. Our unit of reagin is apparently constant.

In titrating the same serum but using different solutions of reagin, identical values for reagin content were obtained. When syphilitic serum was substituted for reagin solution, the readings were likewise unchanged.

The reagin content of the sera of 42 apparently normal individuals was determined. In this small group no significant variation of the reagin titre with sex or age was observed. The results are summarized in Table II.

TABLE II.

No. of Sera	Units of Reagin
27	1.0
12	0.9
2	0.8
1	0.6

The sera of 3 patients with nephrosis and one with myasthenia gravis, all without evidence of syphilis, gave values within the same range. The blood of 6 patients with treated syphilis and with negative Wassermann reactions were also titrated. The results are given in Table III.

TABLE III.

	Units of Reagin
Seronegative primary, treated 6 mo.	0.9
" " " 6 "	1.9
Seropositive " " 2 yr.	1.0
Secondary, treated 1 yr.	1.5
Asymptomatic neurosyphilis, treated 2 yr.	1.0
Taboparesis	1.5

When the serum contained more than one unit of reagin, the determinations were made by the method described for the titration of stock reagin solution.

The results of reagin determinations on various dilutions of the same serum are shown in Table IV.

TABLE IV.

	Reagin Content (Units)	
Dilution	Determined	Calculated
Undiluted	1.0	1.0
%		
25	0.75	0.75
30	0.6	0.5
75	0.35	0.25

The titrated and calculated values agree within 0.1 unit in each dilution. This indicates that the method is accurate over a wide range.

Reagin is evidently present in the sera of non-syphilitic individuals. There is no proof that this reagin is identical with that found in syphilitic sera but it reacts in the same way to the Kline precipitation test. The difference between syphilitic and non-syphilitic sera seems to be a quantitative rather than a qualitative one. Measurements of reagin by the method described here are accurate to about 0.1 unit.

The determination of the reagin content of the serum in the various phases of syphilis and in other diseases, particularly those in which false positives have been reported, is contemplated.

The method described here should provide a direct measure of the sensitivity of serological tests. With a sensitive test, such as the Kline, very small amounts of reagin added to normal serum will give a positive reaction. With an insensitive test, larger amounts would probably be necessary. The amount required should provide an index of sensitivity.

Summary. 1. A method is described for the detection and quantitative measurements of reagin in non-syphilitic sera. 2. Non-

syphilitic sera are regularly found to contain small quantities of reagin.

8314 P

Fenestration of Nuclei of Lymphocytes: A New Diagnostic Sign in Infectious Mononucleosis.

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The abnormality of the nuclei of some of the lymphocytes in infectious mononucleosis described below was first observed in 1933. Since then I have found it in all of the 12 cases of infectious mononucleosis studied and it has not been observed in any other condition, although differential counts are made on about 50 to 100 bloods a day in the laboratory under my direction and the technicians were asked to look especially for these fenestrations. In 2 or 3 instances fenestrated nuclei were observed before the diagnosis of infectious mononucleosis had been made by other methods, and this diagnosis was later confirmed by finding the typical large lymphocytes, a positive Paul and Bunnell test, and by the clinical course.

The fenestrated nuclei* appear at first glance to be nuclei containing multiple nucleoli, but careful inspection shows that in the Wright's stained smear there are actually multiple holes, piercing the nucleus in various directions. In those which are parallel to the light beam through the microscope it is obvious that they are holes; but the majority will, from the laws of chance, not have this direction and their diagonal course through the nucleus gives the appearance of an oval-shaped area of decreased density which may or may not have a clear patch at one end. These may be differentiated from nucleoli by the facts that they are too numerous and that their background is the same color as the rest of the nucleus but paler, while in nucleoli the color is a pure blue with no tinge of red in it.

These fenestrations may be present in the nuclei of either the normal or large lymphocytes but are most often seen in the smallest of the otherwise normal lymphocytes. They are usually present in a relatively small proportion of the lymphocytes and may not be

* Slides and colored illustrations showing these morphologic characteristics were shown in the scientific exhibit at the 85th annual session of the American Medical Association in Cleveland, June, 1934.