membranes through the adsorption of a large amount of protein, is impossible to determine.

Summary. The virus of equine encephalomyelitis, both the Eastern and the Western strains, was found to pass collodion membranes with an average pore diameter of 66 mμ, but was completely held back by those of 60 mμ. The results indicate that this virus has the same filtration end-point, and consequently the same particle size, as that of St. Louis encephalitis, which was shown by Bauer, Fite, and Webster,[8] and Elford and Perdrau[9] to be 20 to 30mμ.

8380 C

Staphylococcal Antihemolysin in Osteomyelitis and Other Staphylococcal Infections.

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The possible clinical application of staphylococcal toxoid in staphylococcal infections makes it necessary to know the amount of circulating antitoxin in the blood of normal individuals, and of persons with staphylococcal infections before treatment with toxoid.

Several recent reports[1-6] have recorded antihemolysin (antitoxin?) values for normal healthy individuals. The earlier reports are not readily comparable because of differences in standards employed in various laboratories. The general conclusion, however, is that sera from normal persons exhibit a wide individual variation of titers. With the establishment of a provisional International Standard for staphylococcus antitoxin,[7] it is possible to render reports in entirely comparable terms. Dolman[3] has reported that the normal value for healthy individuals is from ⅓ to 1 International Unit. Murray[5]

[8] Bauer, J. H., Fite, G. L., and Webster, L. T., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 696.

[9] Elford, W. J., and Perdrau, J. R., *J. Path. and Bact.*, 1935, **40**, 143.

[1] Bryce, L. M., and Burnet, F. M., *J. Path. and Bact.*, 1932, **35**, 183.

[2] Dolman, C. E., *J. Am. Med. Assn.*, 1933, **100**, 1007.

[3] Dolman, C. E., *Lancet*, 1935, **1**, 306.

[4] Parish, H. J., O'Meara, R. A. Q., and Clark, W. H. M., *Lancet*, 1934, **1**, 1054.

[5] Murray, D. S., *Lancet*, 1935, **1**, 303.

[6] Gross, H., *Klin. Wchnschr.*, 1933, **12**, 907.

[7] Hartley, P., and Llewellyn Smith, M., *Quart. Bull. Health Organis., League of Nations*, 1935, Jan., 68.

reported the normal values as ranging from 0.4-0.7 to 2 International Units.

The titration of antihemolysin in staphylococcal infections has, for the most part, been done upon sera of patients with superficial infections, although some sera from cases of osteomyelitis have also been tested. Dolman[2] reported that patients with superficial infections "show no more, and often less, circulating antitoxin than do healthy persons of the same age." In contrast, Connor and McKie[8] found that "patients with superficial staphylococcal infections have in most cases higher titres of antihaemolysin than normal persons." Parish, O'Meara, and Clark,[4] in a few tests of sera from superficial infections, found the same variation in range of titer as in sera from normal persons. The titers obtained by Murray[5] in superficial infections covered the same general range as did the normal sera, with a slightly larger number of higher titers in the group of infections. Murray considered that the difference was not sufficiently great to be of significance.

The general opinion is recorded in the literature that sera from cases of osteomyelitis possess antihemolysin titers definitely above the normal range. Thus, Dolman[3] records titers of 5-6, and occasionally of 10 International Units. Murray[5] records titers averaging 11.7 International Units, and states "there seems general agreement that in osteomyelitis alone is the amount greatly increased."

We have been interested particularly in the staphylococcal antihemolysin content of sera from cases of osteomyelitis, with reference to subsequent treatment of patients with staphylococcal toxoid. This communication records staphylococcal antihemolysin values for a group of patients with osteomyelitis, and for a group of controls, consisting of normal individuals and persons with staphylococcal infections other than osteomyelitis.

The antihemolysin titrations were originally done by determining that dilution of serum which just inhibited hemolysis of 1% rabbit erythrocytes by one M.H.D. of staphylococcus toxin, after incubation for 1 hour at 37°C. Subsequently, a sample of the International Standard Antitoxin was obtained through the courtesy of Dr. Hartley, and our own sera were titrated in terms of the standard. The titers recorded in this paper are in terms of the International Unit.

The toxin used was prepared from the "Ha" strain of *Staphylo-*

[8] Connor, J. I., and McKie, M., *J. Path. and Bact.*, 1933, **37**, 353.

coccus aureus, isolated by Dr. Burky. Our culture was obtained through the courtesy of Dr. Roberts, of the Lederle Laboratories. Cultures were grown in semisolid (0.3%) agar, in an atmosphere of 30% CO_2, for 48 hours at 37°C. The culture was then drained through gauze, and filtered through a Seitz filter. The potency of the filtrate (toxin) was demonstrated by dermonecrotic and lethal tests on rabbits, and by hemolysin tests. It should be emphasized that rabbit erythrocytes must be used in all tests for staphylococcal hemolysin and antihemolysin, as the red blood cells of other animal species have been shown to give inconsistent results[9]—an observation which we have confirmed.

TABLE I.
Staphylococcal Antihemolysin Titers.

	Total No. of Sera	0.5 Internat. Unit or less	1 Internat. Unit	2 Internat. Units	Over 2 Internat. Units
Normal sera	14	12	2	0	0
Wassermann sera	46	42	4	0	0
TB osteomyelitis	4	4	0	0	0
Streptococcal infections	7	4	1	2	0
Staphylococcal infections	25	15	3	3	4
Osteomyelitis	80	39	15	10	16

Table I gives the results of titrations of sera from the various groups studied. The "normal" sera were obtained from persons with no history of immediately preceding staphylococcal infection. The Wassermann sera were taken at random from sera received in the laboratory for the routine Wassermann test. The diagnosis of tuberculous or streptococcal infection was confirmed by the demonstration of the responsible bacteria in smear or culture. Demonstration of the etiologic agent in the staphylococcal infections and in the cases of osteomyelitis was obtained by the isolation of *Staphylococcus aureus* in every instance. The infections, other than osteomyelitis, included boils, furunculosis, cellulitis, lymphangitis, pyemia or septicemia, and infected wounds.

It will be noted that the titer of the great majority of sera from normal persons, or from non-staphylococcal infections, falls within the low range of 1 International Unit or less—well within the range reported as normal by Dolman and by Murray. Only 2 of 71 sera in this group had a titer over 1 International Unit; both sera came from cases of acute rheumatic fever.

In the cases of staphylococcal infections, other than osteomyelitis,

[9] Dolman, C. E., *Canad. Pub. Health J.,* 1932, **23**, 125.

the titer ranged from 0.5 International Unit or less to 22.7 units, with the majority of sera giving a titer of not over 1 International Unit. The titers of 3 sera from cases of boils or furunculosis were 0.5 International Unit. Of 4 sera with titers over 2 International Units, 2 were obtained from cases of septicemia, (4 units) and 2 from wound infections (3 and 22.7 units).

Of the 80 sera from cases of osteomyelitis, 54 gave a titer of 1 International Unit or less, and 25 gave a titer of 2 or more units. One-fifth of the sera had a titer of 3 or more units. Of this latter group, the 2 highest titers obtained were 12.2 and 17.7 units, while the majority ranged around 3 or 4 units. The average titer of this group was 5.1 International Units. It should be noted that all of the cases of primary acute osteomyelitis and most of the acute exacerbations of chronic osteomyelitis are included in this latter group, while of the group with 1 unit or less, all but 7 or 8 were chronic, and the majority had had no recurrence for a considerable period.

It is our opinion, from the results described above, that the "normal" staphylococcal antihemolysin value ranges up to about 1 International Unit. In the case of non-osteomyelitic staphylococcal infections, titers of sera from the majority of cases fall within the normal range, but a few will give titers definitely above normal. These findings appear to confirm, in general, the reports of Dolman, and of Murray.

In contrast to the uniformly high titers obtained in osteomyelitis by Dolman and by Murray, we found that the titer of the sera of about 2/3 of the patients fell within the normal range of 1 International Unit or less, and that the sera of only 1/5 of the patients possessed titers definitely above normal (3 units or more). In view of this, it would appear that the determination of staphylococcal antihemolysin can be of little diagnostic value in bone infections, as has been claimed by Murray.

The fact that low antihemolysin titers are so frequently found in staphylococcal infections does not necessarily indicate inability of the patient to produce antibody. This is shown in a series of about 35 patients with osteomyelitis who were treated with staphylococcal toxoid. In every instance the patient responded to the antigenic stimulus of the toxoid by the development of sera with antihemolysin titers ranging from 3 to 30 times the original titer. The details of this work are to be published later.

Summary. Normal sera possess a staphylococcal antihemolysin titer of about 1 International Unit or less. The titers of sera from our group of staphylococcal infections, other than osteomyelitis,

vary from 0.5 International Unit or less to 22.7 units, with the great majority falling into the normal range. Sera from 3 cases of superficial infections had titers of 0.5 unit.

In contrast to other reported results, about two-thirds of the sera from cases of osteomyelitis had a titer of 1 International Unit or less. Only one-fifth of the sera had definitely high titers, and these, in our series, averaged 5.1 units; the highest was 17.7 units. From our results, it seems to be necessary to modify the conception that a high antihemolysin titer is characteristic of osteomyelitis.

8381 P

Further Studies on Transmissible Myelosis of Mice.*

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It is now well established that lymphatic leukemia of mice is transmissible and neoplastic. Transmission of myeloid leukemia has only recently been accomplished.[1, 2, 3] The transmissible strain previously described from this laboratory[1] originated in a non-irradiated mouse. The basophile myelocytes characteristic of this strain produce myeloid leukemia in some animals, and multiple myeloma in others. Inoculations were successful in 39% of non-irradiated, closely related mice (Stock A) injected with these myeloid cells, and in only 15% of non-irradiated unrelated mice (Stocks R and S). The number of successful transfers was greatly increased by exposing the animals to X-rays prior to inoculation.

The incidence of spontaneous myelosis was approximately 10 times greater among irradiated mice than among their unirradiated siblings.[1] The present communication deals with one of the 2 successful attempts to transmit myeloid leukemia that occurred in uninoculated X-rayed mice. The first strain of transmissible myelosis that was derived from an X-rayed mouse will be referred to as strain Rfb 117. The mouse from which it originated (No. Rfb

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1 Furth, J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 923; *J. Exp. Med.*, 1935, **61**, 423.

2 Parsons, D., *J. Path. and Bact.*, 1935, **40**, 45.

3 Kaalung-Jørgensen, O., *Zeitsch. f. Krebsf.*, 1935, **42**, 393.