

Bovine Mastitis

Notes on Incidence, Aetiology and Diagnosis

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MANY estimates have been suggested as to losses from mastitis in dairy herds. Probably the most interesting is that of Holford⁽¹⁾ who, in 1930, reported a survey of 5000 dairies in New York State in different parts of the Metropolitan milk shed. He showed that 8.6 per cent of the total cows were discarded each year. This did not include reactors to the tuberculin test, nor cows sold for milking purposes. More than half of these cows were discarded on account of udder troubles. There were 1,330,000 cows in New York State, and 4.3 per cent of these would be 57,190. If these cows had remained healthy, Holford estimates that they would have produced 214,442,500 pounds of milk on a basis of 5,500 pounds per cow per year. In his estimate, part of these cows were considered as losing their entire production, and the remainder part of it. The above milk, at \$2.50 per 100 pounds, would have amounted to \$5,361,062. He estimated the decrease in value of each animal due to udder infection at \$50.00, which would be equivalent to \$2,859,500 loss on the animals themselves, and a total loss of \$8,220,562 annually in the State of New York.

Pursuing the idea further, Holford gives the U.S. Department of Agriculture estimate, in 1929, of 21,820,000 cows in the United States. If the percentage of infection were the same, this would be 938,260 animals. He estimated that with average production, based on 4,600 pounds per cow per annum at the lower price of \$1.50 per 100, and \$50.00 decrease in value of each cow, there would be a loss of \$72,011,455 to the industry in one year.

Rosell,⁽²⁾ reporting on work in the Province of Quebec, found that 34.2 per cent of 1,222 cows examined were suffering from mastitis.

Mohler⁽³⁾ refers to the setting aside of \$1,000,000 for a plan of eliminating marked physical cases of mastitis. Physical examinations were made in 3,808 herds containing 94,919 cows. There were 11,683 marked physical cases (12.2 per cent) and 2,263 suspicious cows.

Mohler's figures do not indicate how many of these cows would have been discarded if compensation had not been paid, but they do show that a large number of animals was rapidly approaching that stage.

Udall and Johnson⁽⁴⁾, in 1931, state "No person can estimate the economic loss caused by this disease; probably it exceeds that of either tuberculosis or abortion. In some herds the loss is slight, in others it is distinct, and in many it is severe; each herd pays an unnecessary toll, and the loss is enormous."

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Whether or not there is a noticeable drop in milk yield in chronic cases depends upon the changes which have occurred in the secreting tissue of the mammary gland. Certainly, in this particular, the majority of those cases we have observed have become worse, although some have remained apparently unchanged for more than two years. In some cases, *Streptococcus mastitidis* was recovered from the best milking cows in the herd, and the udders and milk of these cows showed little or no physical change. Such animals, however, were reservoirs of infection from which less resistant animals might contract infection and develop more severe cases of mastitis, with marked injury to the glandular tissue and lowered milk yield.

Whatever the loss may be in those infected cows that show little or no udder change, there is no doubt of the result in well-marked cases of the disease and, while we do not wish to base any estimate on our own figures, which are at present insufficient, or on those from other countries, there is no doubt that the combined loss from depreciation in value of infected cows and lowered milk yield is very considerable.

Streptococcus mastitidis is considered by most workers to be the most common etiological agent. Minett, Stableforth and Edwards⁽⁵⁾ found 82 of 113 unselected cases to be due to streptococci (72.5 per cent) Udall and Johnson⁽⁶⁾ suggest that control of this form would mean elimination of the disease. However, many other bacteria have been reported as etiological factors, including staphylococci, *B. pyogenes* and colon bacilli, to which organisms we shall refer in this paper.

The following work deals with the examination of samples of milk from individual quarters of 594 cows in 28 herds, and 54 samples sent from 34 other herds, from cows obviously affected with mastitis.

Methods of Diagnosis

We shall only refer to the methods we have employed in the examination of these samples, which were: physical examination, appearance of milk, bromthymol blue reaction, rennet coagulation test, microscopic and bacteriological examination.

Collection of samples: Milk from each quarter was collected in a sterile vial, after wiping off the teats with a sodium hypochlorite solution and discarding the first few streams. A separate cloth was used for each cow. Vials were held in a slanted position to the side of the teat to avoid particles of dirt dropping in. They were kept in the refrigerator and examined the following morning.

Physical examination: This was carried out by one of us (H.M. LeG.) as soon as the cows had been milked out. In general the method followed was that outlined by Udall and Johnson⁽⁶⁾.

Appearance of milk: At time of examination the naked eye appearance of the milk was recorded; whether thin, thick, discoloured, flaky, or bloody.

Bromthymol blue reaction: A solution of 0.2 per cent bromthymol blue in 47.5 per cent alcohol was prepared, into which large sheets of filter paper were dipped and allowed to drain. When dry these were cut up into small strips and stored in dry containers. The pH of the solution was adjusted if

necessary so that the papers were yellow after being dipped in normal milk, comparison also being made with the previous lot of papers. Reactions were recorded as dark green, green, light green and yellow.

Rennet coagulation test: The method of Hadley⁽⁷⁾ was employed. This consists of the addition of 0.1 cc. of a 1:50 dilution of cheesemaker's rennet to 5 cc. skim milk and incubation for 1 hour at 37°C. Readings are made at 15 minute intervals. Good milk is firmly clotted at 15 minutes. Milk not clotted at that time but firm at 30 is considered as being probably all right, but later than that is regarded with suspicion. Many samples that appear normal to the eye do not coagulate in 60 minutes. Milk not clotted in this time is recorded as abnormal. We have encountered a few cases in which milk that in other respects appeared normal did not coagulate with this amount of rennet.

Microscopic examination: At first our procedure was to spread 0.01 cc. of the sediment of 10 cc. of milk over an area 1 cm. square. The 4 quarters are placed on one slide which is marked with grease pencil or diamond at one end. One loopful of sediment replaced the measured quantity after counting was discarded and it was not spread over any definite area. When dry the slides are stained with Hasting's stain, of which 0.5 cc. is allowed to remain on the slide for 1 minute, after which 1 cc. distilled water is added, and slides are carefully washed off and dried after 10 minutes. All slides were examined by one of us. (S.H.) Samples of mastitis milk have a tendency to wash off the slides, but we have also had good milk which did not adhere to the glass. Fixation with alcohol did not appear to be of value. Slides were stored in acid alcohol, dried on a clean towel and flamed before use.

Details of the microscopic work are at present being prepared for publication, and in view of this it need only be stated that in experienced hands the microscope is of great value in the rapid diagnosis of mastitis. One can, as a rule, determine if the bacteria came from the udder or were introduced after milking. An increase in leucocytes, except in a recently calved or nearly dry cow, indicates a defensive reaction against bacteria, and with the exception of streptococci, they are usually found inside the leucocytes and not free in the milk. Contaminating organisms free in fresh milk are not picked up by the leucocytes.

Bacteriological examination: Petri dishes of rabbit-blood beef-infusion agar, pH 7.8, were poured and allowed to cool. Two plates were used for each cow, thus allowing one-half plate for each quarter of the udder. One loopful of sediment was spread over this area, which, with properly collected samples, we have found quite sufficient and the plates were incubated for 48 hours at 37°C. Colonies were then fished, examined microscopically and subcultured on blood agar slants, or plain agar, according to indications, and incubated 24 hours. Streptococcus subcultures were seeded in litmus milk, methylene blue milk, sodium hippurate broth, and beef extract broth containing 1 per cent Andrade's indicator and 0.5 per cent of lactose, mannitol, salicin, raffinose, inulin and arabinose. Staphylococci were planted in lactose, maltose, glycerol and mannitol broth and in gelatin. Many more

carbohydrates were employed at first but were discarded as unnecessary. The broth cultures were incubated for a week at 37°C. as a routine procedure, although results could sometimes be obtained as early as 48 hours when required for reporting results. Gelatin stabs were incubated at room temperature for 3 weeks. We followed the outline of Plastridge *et al.*⁽⁸⁾ in interpreting the results obtained with the streptococci. As a rule not many kinds of bacteria were present in a sample, unless there had been gross contamination, but growth was very heavy in some cases of mastitis, while in others it was very slight or there was none at all.

Records: At the time the samples were collected a record of each animal was made. One sheet for each cow is used. This gives herd, ear tag number or other identification, date, history, including age, date of freshening, whether being dried off and any knowledge of previous udder trouble. Lower on the same sheet are spaces to record clinical examination, appearance of milk, and rennet and bromthymol blue tests for each quarter of the udder, and also columns for microscopic and cultural examination. Under the last, only colony appearance and morphology of organisms are recorded, and

TABLE I
Cases of Mastitis in 28 herds of cattle

Herd No.	No. of Cows Milking	Mastitis		Strep. Infections	Staph. Infections	Other Organisms	Cause not Determined	Suspicious	
		No.	%					No.	%
1	108	22	20.4	17	2		3	11	10.2
2	11	3	27.2	1			2		
3	11	2	18.2	2				1	9.0
4	18	15	83.3		14		1	3	16.6
5	37	10	27.0	5	3		2	4	10.8
6	67	61	91.0	57			4	4	6.0
7	12	5	41.6	2	3			1	8.3
8	12	6	50.0		6			1	8.3
9	16	9	56.2	7	2			2	12.5
10	12	3	25.0	2		1*		1	8.3
11	12	3	25.0		3			1	8.3
12	13	10	76.9	10				1	7.7
13	11	5	45.5	4			1	2	18.2
14	11	7	63.6	4	2		1		
15	14	3	21.4	2	1			1	7.1
16	12	4	33.3	1	3			4	33.3
17	11	3	27.2	3				2	18.2
18	13	2	15.4	1	1			1	7.7
19	11	2	18.2		1		1	2	18.2
20	34	9	26.4	7	2			1	2.9
21	15	7	46.6	5	2			2	13.3
22	12	4	33.3	2	2			1	8.3
23	23	7	30.4	4	3			1	4.3
24	42	9	21.4	5			4	2	4.7
25	24	7	29.1	3		1*	3	4	16.6
26	10	2	20.0			1*	1	2	20.0
27	10	7	70.0	7				1	10.0
28	12	5	41.6	5				1	8.3
TOTALS:	594	232	39.0	156	50	3	23	57	9.6

* *B. pyogenes*

all cultures examined are identified by numbers and recorded on separate sheets, one sheet being used for each organism. Animal sheets are filed under their own herd numbers and culture sheets are filed under culture numbers, so that any information regarding the cow or the cultures obtained from it are readily available.

In the first examination of 594 cows in 28 herds, 232 were found to have mastitis, 57 were listed as suspicious and 305 as clean. Particulars of these herds are given in Table 1.

In addition to the cows shown in Table I, samples from 54 cows in 30 other herds were sent in for examination. All these animals had shown definite symptoms of mastitis. Thirty-two were due to streptococci, 6 to staphylococci, 10 to colon bacilli and 2 to *B. pyogenes*. In 4 cases the cause was not determined.

Omitting these 54 animals, and basing calculations on 594 milking cows in 28 herds, 232 or 39 per cent had mastitis and 57 or 9.6 per cent were suspicious. There were 156 cases of streptococcus infection (67.2 per cent of mastitis). Fifty were apparently due to staphylococci (21.5 per cent), 3 were caused by *B. pyogenes* and in 23 the cause was not determined. If 67 per cent of the undetermined cases might be considered as due to streptococci, this would bring the streptococcus cases to 73.7 per cent of the total mastitis cases.

Grouping the two lots, there are 286 of mastitis, 185 of which were due to streptococci (64.7 per cent), and of these 160 or 86.5 per cent were *Streptococcus mastitidis*. There were 56 or 19.6 per cent of staphylococcus infections, 10 colon (3.5 per cent) and 5 *B. pyogenes* infections (1.7 per cent). In regard to the staphylococcic infections, it should be pointed out that if herds Nos. 4 and 8 were withdrawn there would be 264 cases of mastitis, 36 or 13.6 per cent of which were due to staphylococci. As only 2 of 28 herds showed any considerable number of staphylococcic cases this is probably a truer figure for this infection.

Comparison of Diagnostic Methods

The following comparisons are based on the first examination only. There is no doubt that repeat tests would have reduced the percentage of error in all the tests, as was found to be the case in those herds that have been regularly retested.

Cultural methods failed to demonstrate the causal organism in 26 of 286 definite cases of mastitis (9.1 per cent).

The bromthymol blue and rennet coagulation tests both failed in 73 or 25.5 per cent of these cases. Considered separately, the efficiency of these two tests was about the same. Bromthymol blue failed in 88 cases and the rennet test in 86, or approximately 30 per cent for both.

Clinical examination of 594 cows failed to detect 48 or 20.7 per cent of the 232 cases of mastitis diagnosed by other methods, or in other words, this percentage of cattle did not show lesions we were able to detect. Thirty-three of these cases were due to streptococci and the others to staphylococci, therefore there was failure to detect physical evidence of infection in the

udders of 33 of 156 streptococcus infections (21.2 per cent) and in 15 of the 50 milder staphylococcus cases (30 per cent). Eleven cows with indurated mammary glands appeared normal by all other tests (1.8 per cent). It is not unreasonable to assume that these were recovered or latent cases.

In 200 cases from which *Streptococcus mastitidis* was recovered by cultural methods, the microscope showed 15 where there were few cells and no cocci seen (7.5 per cent), and 35 cases in which there was an abnormal cell picture but no cocci were observed (17.5 per cent).

Incidence of Mastitis in Bovine Primipara

It is generally agreed that the incidence of infection in heifers is lower than in older animals, and we have found such to be the case in the 135 animals in this class that we have examined. One hundred and eleven of these animals were normal by all tests (82.2 per cent), ten were classified as suspicious (7.4 per cent), and 14 had definite cases of mastitis (10.4 per cent). Ten of these were streptococcus infections and 4 were apparently due to *Staph. aureus*. Of these last, 2 were infected in the right-front and 2 in left-hind quarters. Two of the streptococcic cases were infected in the right-front, 2 in right-hind, 1 left-front, 1 left-hind, 1 left-side, 1 in 3-quarters and 2 in all quarters.

One of these animals was examined 2 weeks after freshening and at this time the milk of the affected quarter gave a dark green reaction by the bromthymol blue test and did not coagulate with rennet. One of the staphylococcus cases occurred shortly after freshening, and may have been directly connected with an injury, while another was being dried off.

In the 594 milking animals in the 28 herds, 135 were in their first lactation period and the percentage of infection was 10.4 compared to 47.5 for the remaining 459 animals in which were 218 cases of mastitis. While the incidence of infection is much lower in the young animals, yet it represents a definite proportion of the loss in these herds.

Numbers of Quarters Affected

In an examination of 265 cases of mastitis, including the 14 heifers in the previous section, and based on all the tests employed, it was found that the largest number was infected in all quarters. Infection by quarters was found to be as follows: Twenty-one were infected in right-front quarter only, 24 in right-hind only, 9 in left-front only, 25 in left-hind only, 10 in both hind quarters, 10 in both front quarters, 12 in right-side only, 8 in left-side only, 18 in 2-diagonal quarters, 44 in 3-quarters and 84 in all quarters.

Considering single quarters only, there were 49 hind-quarter infections compared to 30 front-quarters. The numbers, however, are too small to be more than suggestive.

Munch-Petersen⁽⁹⁾ cites four authors regarding the average number of diseased quarters. Among these Seeleman⁽¹⁰⁾ shows that the largest number of animals had 4 quarters infected. His figures are:—Four quarters infected 27.9 per cent, 3 quarters 12.5 per cent, 2 quarters 12.5 per cent, 1 quarter 13.8 per cent. Others show more cows with infection in one quarter only. It is obvious that the age of the animals at time of examination would be a

factor in the number of quarters infected. Seeleman concluded that no one quarter was particularly subjected to infection.

Nottbohm⁽¹¹⁾ in 18 cows found the left-front quarter attacked in 61 per cent of the cases, right-hind in 22 per cent and right-front in only 1 case. This was ascribed to the fact that cows usually rest on the left side. Apparently only the chlorine and lactose tests were employed.

Staphylococcic Mastitis

We have reported our findings in Staphylococcic mastitis in two previous papers.^(12, 13) It was pointed out that in addition to occurring in sporadic cases, as has been recognized for a long time, this type of infection should also be regarded as a possible herd infection, occurring in a chronic form and giving rise to lesions similar to those in the streptococcic form of mastitis. The work of Minett⁽¹⁴⁾ supports this view, although he considers some of the staphylococci examined were not the causative agent as they did not produce toxin. Fourteen of 18 cows in one herd were affected, the milk was abnormal by the bromthymol blue and rennet tests and the udders of 12 were indurated. No streptococci were isolated at any time in this herd. As has already been pointed out, the high incidence of staphylococcus infection in 2 of the 28 herds accounts for the high percentage of this infection in this series (19.6 percent). Without these, the percentage in the other 26 herds would be 13.6 which, in view of the fact that only 2 of 28 herds showed any considerable number of staphylococcic cases, is probably a truer figure for this infection.

Colon Mastitis

Mastitis due to organisms of the colon group has been reported by different workers. Among the more recent publications Minett *et al.*⁽⁵⁾ report 3 cases due to diverse organisms belonging to the colon group. One was very severe and the other two were mild clinically. Lesbouyries and Renaudon⁽¹⁵⁾ report the frequent occurrence of a paraplegic colon mastitis in certain regions of France. A hind quarter was usually affected and the other quarters rapidly dried up. Hupka⁽¹⁶⁾ also reports this form of mastitis, which he found to attack the hind quarters only. The mammitis and paralysis of hind limbs come on so quickly that the milker does not notice the former but considers the latter the primary condition. Lesbouyries⁽¹⁷⁾ points out that colon mastitis may appear in several forms, non-paraplegic, in which lesions may sometimes extend to the front quarters and the paraplegic type, in which the right-hind quarter seems to be mostly attacked. Frequently a passing and fetid diarrhoea precedes the attack.

Ten of the 286 cases of mastitis recorded in this paper were apparently due to members of the colon-aerogenes group (3.5 per cent). All were acute, 6 terminated fatally, 1 made a complete recovery, 1 recovered with loss of the affected quarter and 2 recovered sufficiently to be sold for slaughter. While the number is small, there are certain interesting features which justify further study and work is being continued along these lines.

B. pyogenes Infection

Infection of the udder with *B. pyogenes* has been reported by various workers. Minett *et al.*⁽⁵⁾ report 12 cases. One animal died, and in most of the remainder the affected quarter was reported to have lost its function.

We encountered 5 cases, particulars of which are as follows: No. 1. Sample received in April. An old cow. Dry. All quarters very hard and lumpy. Secretion was a clear yellowish fluid with a brown clot and gave a dark green reaction with bromthymol blue. Microscopically there was a marked increase in polymorphonuclear leucocytes in all quarters. A pure culture of *B. pyogenes* was obtained from three, while from the fourth *Staph. aureus* was also isolated.

No. 2. Sample received in April. This cow was in the same stable as No. 1, and was almost dry. All quarters were severely indurated and the secretion of all was abnormal, ranging from a flaky, dirty looking milk to a clear, thin fluid with a heavy, yellowish-brown, flocculent sediment. Microscopically the milk showed a great increase in polymorphonuclear leucocytes in 3 quarters, while mononuclear cells predominated in the fourth. *B. pyogenes* was isolated from 3 quarters with only a few coarse coccus forms, and a streptococcus, not *mastitidis*, was also recovered.

No. 3. A 7-year old Holstein. Calved December 14th and developed mastitis in right-hind quarter on December 18th. Sample was collected the next day, at which time the cow was showing marked constitutional disturbance. Physical examination showed all quarters to be apparently normal except the right-hind which was badly swollen. The secretion of this quarter was a dirty, greenish-yellow fluid which, on microscopic examination, showed very few white cells, degenerate red blood cells and a great number of small bacilli. On blood agar plates, there was a heavy growth of fine colonies which were identified as *B. pyogenes*. The cow rapidly became worse and died a few days later. There were 2 cases of streptococcic mastitis in the remaining 11 animals in this herd but *B. pyogenes* was not again encountered.

No. 4. A 3-year old Holstein. Had freshened 4 months. In January a few drops of fluid were obtained from the right-hind quarter which was dry. A mixed culture of *B. pyogenes* and *Staph. aureus* was obtained. The right front quarter was indurated and the left side appeared normal. No bacteria of significance were obtained from these quarters. Microscopic examination showed complete breaking down of cells in the infected quarter.

No. 5. Aged Holstein. Had freshened about 3 weeks. Right-front quarter was indurated and contained a small quantity of a greenish-brown fluid which on culture yielded a pure growth of *B. pyogenes*. Microscopic examination showed the usual picture of degenerate white cells and great numbers of small bacilli. This was a case of long standing. The other quarters appeared to be normal.

Summary

Five hundred and ninety-four cows in 28 herds were examined physically, and the milk of individual quarters was submitted to the bromthymol blue and rennet tests and microscopic and bacteriological examination.

Two hundred and thirty-two or 39 per cent of these animals had mastitis, 57 or 9.6 per cent were suspicious and 305 or 51.4 per cent were free from evidence of infection. There were 156 cases of streptococcus infection (67.2 per cent of cases of mastitis), 50 staphylococcus (21.5 per cent), 3 *B. pyogenes* and 23 in which the cause was not determined. If 67 per cent of cases of undetermined etiology were added to the streptococcus cases that figure would be about 73.7 per cent of the total cases. The percentage of infected animals ranged from 15 to 91 in the 28 herds.

In addition to these, 54 samples were sent in from 30 other herds, Thirty-two of these were due to streptococci, 6 to staphylococci, 10 to members of the colon-aerogenes group, 2 to *B. pyogenes*, while in 4 the cause was not determined.

Grouping the two lots, there were 286 cases of mastitis, 185 of which were due to streptococci (64.7 per cent), or if 67 per cent of the undetermined cases be considered as streptococcic this percentage would be about 71, and of the streptococci examined 86.5 per cent were *Streptococcus mastitidis*. There were 56 staphylococcus infections (19.6 per cent) but as 20 of these cases occurred in 2 herds, the 13.6 per cent encountered in the remaining 26 herds is probably a truer figure for this infection. There were 10 colon (3.5 per cent) and 5 *B. pyogenes* infections (1.7 per cent).

Cultures failed to demonstrate the causal organism in 9.1 per cent of the cases of mastitis. Bromthymol blue and rennet coagulation tests together were negative in 25.5 per cent of these cases. Clinical examination failed to pick 20.7 per cent of cases determined by the combined methods. Microscopic examination, in 200 cases in which *Streptococcus mastitidis* was isolated, showed 15 cases where there were few cells and no cocci (7.5 per cent) and 35 in which there was an abnormal cell picture but no cocci were observed (17.5 per cent). These figures are based on one examination only. If subsequent examinations were included the percentage of error would be less, as shown by results in those herds that have been regularly retested. Eleven cows with indurated mammary glands appeared normal by all other tests.

The percentage of infection in primipara was 10.4 compared to 47.5 in the older animals.

Of 265 cases 31.7 per cent were infected in all quarters. Considering single quarters only, there were 49 hind-quarter infections compared to 30 front quarters.

Ten cases of infection due to organisms of the colon-aerogenes group were encountered (3.5 per cent). All were acute and six terminated fatally.

Five cases of *B. pyogenes* infection were diagnosed (1.7 per cent). Four were chronic and one was acute with a fatal termination.

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Antibacterial Immunity to *Staphylococcus Pyogenes*

WITH regard to *Staphylococcus Pyogenes*, many workers have shown that a staphylococcal bacteraemia often develops and may terminate fatally in man or animals possessing a considerable amount of staphylococcus antitoxin in their blood. Lyons⁽¹⁾ has presented evidence to show that young cultures of toxigenic and non-toxigenic strains of *Staphylococcus Pyogenes* are capsulated and that phagocytosis of these young cultures is not so rapid as with old cultures. He claims that phagocytosis of capsulated staphylococci will eventually take place but that toxigenic strains are capable of becoming re-encapsulated within the leucocyte, killing the leucocyte and returning to the blood stream. Immunization of rabbits with heat killed encapsulated organisms gives rise to a type-specific anti-capsular antibody which can be detected by slide agglutination using the homologous antigen. This antibacterial antibody will so sensitize toxigenic encapsulated organisms that they are easily phagocytosed and destroyed. Rabbits containing this type specific antibacterial antibody as a result of immunization will effectively clear their blood stream of intravenously injected staphylococci whereas normal controls and rabbits possessing antitoxic immunity fail to do so. Lyons fully recognizes the importance of antitoxic immunity in staphylococcal infections but feels that therapeutic sera should contain both antibacterial and antitoxic antibodies.

¹Lyons Champ—*Brit Jour. Exp. Path.* Oct. 1937, Vol. XVIII, No. 5.