they were not infected on the same days as the monkeys in Experiments 1 and 2. These animals may also be considered controls since Coggeshall and Eaton[8] demonstrated that the number of parasites introduced influences only the length of the prepatent period. Once the parasites become demonstrable, the progress of the disease is almost uniform, with death occurring usually on the 3rd to 6th day of the parasitemia. Table 2 shows that in the unimmunized animals parasites were first found in thin blood smears from 3 to 8 days after infection, and thereafter multiplied rapidly. Four of the 14 monkeys died with acute malaria. Nine monkeys were killed (to recover parasites for vaccine production). One unimmunized monkey survived after an infection which at its peak showed 8 parasites per 100 r.b.c. Mulligan and Sinton observed that 1 of 120, and Coggeshall and Kumm[5] 1 of 70 monkeys recovered spontaneously from an infection with *P. knowlesi*.

TABLE 3

PARASITEMIA IN VACCINATED MONKEYS
PARASITES PER 100 RED BLOOD CELLS

Days after infection	Unconcentrated vaccine			Monkey number	Concentrated vaccine		
	15	16	17	18	19	20	21
1	0	0	0	–	–	–	–
2	0	0	0	0	0	0	0
3	0	0	0	–	–	–	–
4	0	0	0	0	0	0	0
5	0	0	.1	0	0	0	0
6	÷	0	.1	0	0	0	0
7	1	0	.2	0	0	0	0
8	10	0	3D	0	0	0	0
9	4	0		0	0	0	0
10	8	.1		0	0	0	0
11	5	0		.1	0	.1	.1
12	6	.6		.4	0	.4	0
13	2	2		.5	0	1	.2
14	.7	2		.5	0	.6	1
15	.3	1		2	0	1	.5
16	.1	.4		2	0	.6	.6
17	.2	0		.5	0	.3	.1
18	.1	.1		2	0	.4	0
19	.2	0		6	0	.7	0
20	0	0		3	0	.3	0
21	.1	0		.2	0	.2	0
22	0	0		0	0	0	.1
23	.2	0		0	0	0	0
24	÷	.1		–	–	–	–
25	0	0		0	0	0	0
26	.1	0		0	0	0	0

Experiment 1: Monkey Nos. 15, 16, 17.
Experiment 2: Monkey Nos. 18, 19, 20, 21.
Monkey No. 15 died with extensive tuberculosis on the 69th day after infection. From the 27th to the 68th day it was examined for parasitemia on 21 different days. Parasites were found on 9 days, being 1 in 1,000 or more r.b.c. On the 68th day, one parasite was found in more than 1,000 r.b.c.
Monkey No. 16 died of tuberculosis on the 49th day after infection. From the 27th to the 49th day it was examined for parasitemia on 15 different days. No parasites were found.
Monkey No. 17 died in acute respiratory distress while a blood smear was being taken. Nine tenths of the cut surface of the lungs were involved in tuberculosis.

Table 3 shows the parasite counts in immunized animals. In one of 7 monkeys parasites were not found in thin blood smears. In the other six animals there were only 1, 1, 2, 3, 6 and 10 parasites per 100 r.b.c. at the peak of infection. Subsequently the parasites decreased until none were demonstrable in thin smears.

Although the number of monkeys in the experiments was small the difference between the course of infection in vaccinated and non-vaccinated monkeys seems significant considering the high virulence of *P. knowlesi* for the Rhesus monkey.

Palpable masses were found in the subcutaneous tissue at the sites of injection in all vaccinated monkeys. These masses did not ulcerate through the skin in the three monkeys which showed no reaction to tuberculin (P.P.D.). However, it may be noted that one of these monkeys (No. 15) showed extensive tuberculosis at autopsy 85 days after its negative reaction to P.P.D. Abscess formation and ulceration through the skin were observed in the four monkeys which reacted to P.P.D. Two of these animals died and showed extensive tuberculosis at autopsy.

CONCLUSION

The injection of formalin killed *P. knowlesi* parasites combined with a lanolin-like substance and paraffin oil containing killed tubercle bacilli modifies parasitemia and prevents fatal infection with *P. knowlesi* in Rhesus monkeys.

JULES FREUND
K. J. THOMSON
HARRIET E. SOMMER
ANNABEL W. WALTER
EDNA L. SCHENKEIN

PUBLIC HEALTH RESEARCH INSTITUTE
OF THE CITY OF NEW YORK, INC.
AND THE DEPARTMENT OF HEALTH,
BUREAU OF LABORATORIES,
CITY OF NEW YORK

SCIENTIFIC APPARATUS AND LABORATORY METHODS

METHOD FOR THE DETECTION OF INDOLE[1]

THE detection of indole is a matter of importance in a number of different fields. For instance, principal uses of this test are (1) the differentiation of bacteria, some of which characteristically produce indole while others do not[2]; (2) the detection of spoilage of foods[3]; (3) the demonstration of the

[1] Contribution from Department of Health, City of New York.

[2] Am. Pub. Health Assoc. Standard Methods of Water Analysis. 8th ed. 1936.

[3] Clarke, Cannon, Coulter, Goodman, Greene, Milsted, Vandaveer and Wildman, *Jour. Assoc. Official Agr. Chem.*, 20: 475, 1937; *Chernoff, Ind. Eng. Chem.*, Anal. Ed., 12: 273, 1940.

presence of indole producing organisms in milk,[4] food and water as evidence of pollution; and (4) detection and determination of indole in blood,[5] urine,[6] and feces[7] for clinical diagnosis.

The most widely used test for the detection and determination of indole is its reaction with *p*-dimethylaminobenzaldehyde. This reaction, introduced by Ehrlich[8] and modified by Kovács,[9] has achieved official standing by being adopted by the American Public Health Association as one of the tests for the differentiation of *E. coli* from *A. aerogenes*. This direct reaction, however, is not specific for indole, for an entire series of compounds, such as aldehydes, also give colored compounds, nor is it sufficiently sensitive.[10] Because of this lack of sensitivity it is necessary to isolate the indole before applying the test reaction. This has been done by extraction with immiscible solvents, by steam distillation and by dialysis.

p-Dimethylaminobenzaldehyde-trichloroacetic Acid Test

The following method, which can detect directly 1 microgram of indole in 10 ml of test solution or 1 part of indole in 10 million of solution or by an extraction modification can detect 1 microgram in 100 ml of test solution or 1 part per 100,000,000, is relatively simple, rapid and sensitive.

Reagents: (A) Dissolve 10 grams of trichloroacetic acid in 30 ml of chloroform. Add 2 grams of *p*-dimethylaminobenzaldehyde. Transfer to a separatory funnel and saturate by shaking with concentrated hydrochloric acid. This reagent is stable for at least 2 months. (B) Dissolve 10 grams of trichloroacetic acid in 30 ml of chloroform. Add 2 grams of *p*-dimethylaminobenzaldehyde. Add 0.5 ml of acetic anhydride and 2 drops of concentrated sulfuric acid. This reagent is stable for at least 2 days. It is more sensitive than the reagent (A) above.

Detection: Transfer 10 ml of the culture or 10 ml of the sample solution to a separatory funnel. Add 2 ml of chloroform. Shake vigorously and allow to stand for 5 minutes. Swirl to make sure the chloroform layer collects at the bottom. Filter through a filter wetted with chloroform into a small test-tube or insert a pledget of cotton into the stem of the separatory funnel and draw off the chloroform layer directly into a test-tube. Add 2 drops of either reagent listed above and hold in warm water (50° C.) for 0.5 minute. A pink, to rose or red, color is positive for indole. A straw color or light yellow is negative for indole.

Instead of the chloroform extractant, 2 ml of a mixture of equal volumes of chloroform and carbon tetrachloride may be used.

Determination: Shake out twice with chloroform or with the mixed solvent. Filter into a colorimeter tube or a 10 ml volumetric flask as directed above. Make up to volume with the mixed solvent or chloroform and compare against standards treated the same way.

Discussion: Only for very low concentrations of indole is the chloroform extraction necessary. In most instances, especially in cultures after 24 hours incubation, the reagents may be added directly to the culture to be tested. The color forms at the interface or is extracted by mild shaking and is found in the bottom chloroform layer.

The addition of 5 ml of acetic anhydride to reagent (A) does not seem to alter its properties but if acetic acid is used instead of hydrochloric acid, it does not give the reaction. Dilution with half its volume of carbon tetrachloride reduces its sensitivity. Reagent (B) without acetic anhydride will work only if freshly prepared. It deteriorates rapidly.

With low concentrations of indole of the order of 1 to 0.1 micrograms per ml of chloroform, the pink color fades out rather rapidly with reagent (A). With higher concentrations, the color does not fade out even on standing 24 hours. The color formed with reagent (B) does not fade even after standing for hours. When carbon tetrachloride is used as part of the solvent mixture, the color comes up fast but fades out rapidly. It lasts longest with reagent (B) but is weak in intensity.

The reagent is affected by water, consequently when it is shaken with a culture or with aqueous solutions it loses in sensitivity. For greatest sensitivity it is necessary to use the extraction method as detailed above.

Experimental: The solutions of indole were prepared by dissolving 1.00 gram of indole in 95 per cent. alcohol, transferring to a 100 ml flask and diluting to volume with the same solvent. Suitable aliquots were withdrawn and diluted with water in volumetric flasks so that concentrations of the order of 0.01 to 1 microgram per ml of test solution were obtained. These solutions were used to establish the order of sensitivity mentioned previously.

The bacteriological method was tested on 167 cultures of which 46 were *E. coli* cultures (10 specimens), 8 were salmonella cultures (2 specimens), 7 were Sonné cultures (1 specimen), 4 were aerogenes cul-

[4] Damm and Bartram, Molkerei-Ztg. (Hildesheim) 50: 714, 1936; *Chem. Abstracts,* 30: 6836, 1936.
[5] Mazzocco, *Rev. soc. argentina biol.,* 11: 31, 1935; *Compt. rend. soc. biol.,* 119: 699, 1935.
[6] Vaughan, *Proc. Soc. Exp. Biol. and Med.,* 29: 623, 1932.
[7] Bergeim, *Jour. Biol. Chem.,* 32: 17, 1917.
[8] Ehrlich, *Deut. med. Wochschr.,* 1901, 1.
[9] Kovács, *Z. Immunitäts.,* 55: 311, 1928.
[10] Lehr, *Centr. Bakt. Parasitenk.* Orig. I Abt. 108: 209, 1928.

tures (1 specimen), 84 were cultures of sewage (6 specimens), and 22 were uninoculated blanks.

Regulation methods were used for the inoculation of a tryptone culture medium. Cultures were withdrawn from incubation in the first experiments at the end of 1, 2, 3, 4, 5, 6 and 24 hours and were tested for indole. Later experiments on cultures were performed after incubation at 37° C. after 2, 3, 4 and 24 hours.

Results: The inoculated specimens of *E. coli* yielded positive results in 7 out of 8 cases at the end of 3 and 4 hours incubation and in the 8th at the end of 24 hours incubation. However, 2 specimens were negative throughout. Four out of 8 were positive for indole at the end of 2 hours incubation. Mastafa[11] noted that a medium made by peptic digestion of entire hogs' stomachs could be used for the detection of indole at the end of 2 hours incubation. The Sonné, salmonella and aerogenes specimens were negative for indole production as expected. Except for trace results, the experiments with raw sewage, and treated sewage, obtained from a large city sewage treatment works, diluted 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000 and undiluted, were negative or at best yielded a trace result for indole, at the end of 5 hours incubation, with the exception of two undiluted raw sewage specimens which yielded positives at the end of 5 hours incubation. However, every dilution of raw sewage yielded a strong positive at the end of 24 hours. Partially treated sewage yielded positives in dilutions up to 1:100 and settled sewage only when undiluted.

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SUMMARY

A simple and sensitive method for the detection of indole in cultures, urine, sewage, etc., is presented. The indole is extracted with chloroform and is then treated with a modified Ehrlich reagent. The possibility of use for the detection of water pollution is indicated.

MORRIS B. JACOBS,
Senior Chemist and Director, Gas Reconnaissance
SOL PINCUS,
Deputy Commissioner of Health and Sanitary Engineer

AN INSTRUMENT FOR THE RAPID MIXING OF FLUIDS IN SMALL TUBES[1,2]

IN the preparation of serial dilutions for purposes such as the titration of viruses and immune sera, the material in question is transferred to a tube containing diluent. Mixing is usually then effected by drawing the fluid to and fro in a pipette several times before transfer is made to another tube for the next dilution. When many dilutions are to be made, this process of mixing is not only time-consuming but physically wearing. In the contemplation of a large series of studies[3,4] on the titration of serum antibodies inhibiting the hemagglutinative action of the influenza virus,[5] a simple means was found for obviating much of the tedium and loss of time. This consists in the use of an electric massage vibrator[6] of the type shown in Fig. 1, which is a drawing taken from a photograph. The motor mechanism is mounted inside a closed metal chassis from which there projects a short rod. The rod is the vibrating part, and for it there are provided several rubber attachments. The best of these for the purpose is the bell-shaped one, illustrated in Fig. 1. The instrument is fixed to the table with an iron clamp, set going and the tube containing the materials to be mixed is held against the edge of the bell. As shown in Fig. 1, the mixing action is highly effective. Some trial is necessary, however, for finding the best point and the proper angle for application of the tube.

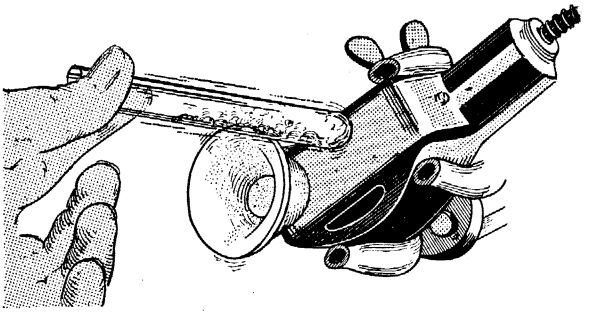

FIG. 1. A drawing of the vibrator and the mixing of fluid in the tube based on the tracing of a photograph.

Repeated tests have shown that mixing as good as that with a pipette can be obtained by holding the tube against the machine with the left hand during the period in which the right hand is selecting the

[11] Mastafa, *Compt. rend. soc. biol.*, 124: 450, 1937.

[1] From the Department of Surgery, Duke University School of Medicine, Durham, N. C.

[2] This work was aided by the Commission on Influenza and the Commission on Epidemiological Survey, Board for the Investigation and Control of Influenza and Other Epidemic Diseases in the Army, Preventive Medicine Service, Office of the Surgeon General, United States Army. The work was aided also in part by a grant to Duke University from Lederle Laboratories, Inc., Pearl River, N. Y.

[3] I. W. McLean, Jr., D. Beard, A. R. Taylor, D. G. Sharp and J. W. Beard, *Jour. Immunol.*, in press.

[4] *Idem.* In preparation.

[5] G. K. Hirst, *Jour. Exp. Med.*, 75: 49–64, 1942.

[6] Allover A.C. Vibrator, Allover Mfg. Co., Racine, Wis.