TABLE III.
Effect of Body Fluids and Pus on the Action of Streptomycin *in Vitro*. Incubation 48 hr.

Medium	Growth by visual turbidity. Units of streptomycin per cc							Growth by subculture.‡ Units of streptomycin per cc					
	1	2	3	4	5	7.5	10	2	3	4	5	7.5	10
Broth (control)	G*	0	0	0	0	0	0	+†	+	+	—	0	0
Broth + purulent pleural fluid	G	0	0	0	0	0	0	+	—	+	—	0	0
Broth + non-purulent pleural fluid	G	G	0	0	0	0	0	—	+	+	—	0	0
Broth + ascitic fluid	G	G	0	0	0	0	0	+	—	+	—	0	0

* G indicates visual turbidity.
† + indicates growth on subculture; 0 indicates no growth; — indicates tube not subcultured.
‡ One double loopful streaked on surface of agar plate.
Inoculum: 0.1 cc 1:10 dilution of 12-hour broth culture of *Staphylococcus aureus* Smith.

aureus possess a natural increased resistance to streptomycin. This resistance is not based on the production of a "streptomycinase." 2. A strain of staphylococcus, highly resistant to streptomycin, can be produced *in vitro* from the streptomycin-sensitive organisms. 3. Streptomycin resistance is a relatively permanent characteristic, and is not affected by several passages through mice. The resistant organisms retain their virulence for this host. 4. Streptomycin resistance and penicillin resistance are independent of each other. Staphylococci originally penicillin-sensitive, retain their sensitivity to penicillin after being made resistant to streptomycin, and vice versa. Treatment with relatively large amounts of streptomycin is ineffective in mice infected with a streptomycin-resistant strain of *Staphylococcus aureus*. These mice can be cured, however, with penicillin. 5. The bacteriostatic effect of streptomycin diminishes considerably as the acidity of the culture medium increases from pH 7.7 to 5.2; the greatest diminution in effect occurs between pH 6.6 and 5.9. This must be taken into consideration in making *in vitro* assays and sensitivity tests, as well as in evaluating the action of the drug *in vivo*. 6. Streptomycin is not destroyed after contact with broth of pH 3.5 for 2½ hours. 7. Streptomycin is not destroyed, nor are its bacteriostatic and bactericidal powers appreciably influenced, by serous body fluids, pus, or normal tissue juices.

15408

Electrophoretic and Allergenic Analyses of Fractions of Larvae of *Trichinella spiralis*.

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Fractions of larvae of *Trichinella spiralis* have been employed as diagnostic aids in detecting *Trichinella* infection in man. Bachman[1] used an acid-hydrolyzed, saline-soluble extract of *Trichinella* larvae to study intradermal responses in sensitized rabbits. Bozicevich[2] prepared an antigen for precipitin tests from the supernatant fluid of a 5% saline extract heated at 58°C for one hour to precipitate inert material; the same material, diluted and sterilized by intermittent

[1] Bachman, G. W., *J. Prev. Med.*, 1923, **2**, 169.

[2] Bozicevich, J., *Pub. Health Rep.*, 1938, **53**, 2130.

heating, was used without a preservative as an allergenic agent. Melcher[3] separated 6 fractions of larvae and found the acid-soluble fraction to be a potent allergen. This fraction was prepared by alkaline extraction of dehydrated, defatted larvae from which the acid-insoluble non-reactive substances had been removed.

In the present study a comparison was made of electrophoretic and allergenic properties of acid-soluble and of heat-treated fractions with those of the parent saline extract of *Trichinella* larvae. An electrophoretic analysis was made of each fraction in the modified Tiselius apparatus by the moving boundary method.[4] The allergenic properties of the fractions were compared in sensitized rabbits and guinea pigs.

Procedure. Obtaining larvae. About 5,000 larvae suspended in 20% gelatin were fed to each guinea pig by means of a syringe connected to a rubber catheter. Approximately 7 weeks later, the animals were killed and the skinned and eviscerated carcasses were ground twice in a meat chopper. The muscle was digested by the method described by Bozicevich[2] and modified by Line.[5] Approximately 150 g of ground material were placed in a 2 liter beaker and covered with warm digestion fluid which contained 2.5 g of pepsin (activity 1:3,000) and 7 ml hydrochloric acid (sp. gr. 1.18) in one liter of water. Twelve thicknesses of 44 mesh gauze were tied around the top of the beaker. A funnel of 2 liter capacity was connected to a centrifuge tube with rubber tubing. The funnel was almost filled with the digestion fluid. The beaker containing the muscle was covered with a piece of paper and quickly inverted into the funnel. At this point, the beaker rested in an inverted position with the level of the digestion fluid in the funnel above the rim of the beaker. The paper was withdrawn carefully to avoid unnecessary stirring. As digestion proceeded at 37°C for 5-7 hours, the larvae settled to the bottom of the centrifuge tube. The freed larvae were alternately washed with distilled water and centrifuged 6 times, frozen at —12°C, dried under vacuum from the frozen state and sealed under vacuum.

Extraction. A weighed amount of dried larvae was ground in sterile 0.85% salt solution in a sterile tissue grinder and diluted to a 5% suspension. The material was held overnight at 4°C, adjusted to pH 7.0 and centrifuged at 3,500 r.p.m. (2,200 × gravity calculated from the radius at the base of the angle head). The supernatant fluid is designated as the primary extract.

Acid treatment. One portion of the extract was adjusted to pH 4.8 with 0.2 N hydrochloric acid in a Beckman pH meter and held at 4°C overnight. Insoluble material was removed by centrifugation and the supernatant fluid designated as the acid-soluble fraction. The sample for intradermal injections was adjusted to neutrality with 0.1 N sodium hydroxide. This fraction was not exactly comparable to the acid-soluble fraction analyzed by Melcher.[3]

Heat treatment. A second portion of the extract was heated at 56°C for an hour. This was likewise centrifuged and the supernatant fluid referred to as the heat-treated fraction.

Electrophoretic technic. For electrophoretic analysis the samples were placed in viscose tubing and dialyzed at 3°C against large volumes of phosphate buffer of pH 6.8 and ionic strength 0.03. Electrophoresis was carried out at 0°C and the schlieren scanning method of Longsworth[6] was used in photographing the boundaries.

Asymmetric boundaries were obtained in all patterns. Since the ascending boundaries swept through larger volumes and a better separation of components was obtained than in the corresponding descending boundaries, all calculations were made from ascending patterns. In computing relative areas it was assumed that the immobile peaks in all patterns were δ and ϵ boundaries.

Intradermal tests. Infected rabbits and guinea pigs were used for comparing allergenic potencies. The rabbits had been fed about

[3] Melcher, L. R., *J. Inf. Dis.*, 1943, **73**, 31.

[4] Longsworth, L. G., and MacInnes, D. A., *Chem. Rev.*, 1939, **24**, 271.

[5] Line, C. B., unpublished procedure.

[6] Longsworth, L. G., *Ann. New York Acad. Sc.*, 1939, **39**, 187.

Fig. 1.
Primary extract of larvae of *Trichinella spiralis*. Sample diluted 1-2.7. Electrophoresis for 3225 seconds at 8.87 volts per cm.

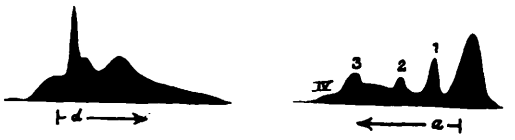

Fig. 2.
Acid-soluble fraction. Electrophoresis for 5600 seconds at 7.73 volts per cm.

Fig. 3.
Heat-treated fraction. Electrophoresis for 5700 seconds at 8.81 volts per cm.

20,000 larvae each, 4 to 16 months prior to test, the guinea pigs about 5,000 larvae each, 18 months previously. For testing, samples of the 3 fractions were filtered through a sterile, fine porosity fritted-glass filter and diluted. A sterility test on each sample was satisfactory. Dilutions of 1:10,000 based on the original dry weight of whole larvae were made for injections into rabbits and 1:1,000 for guinea pigs. Animals were injected intradermally with 0.05 ml and observed hourly. The maximum reaction usually occurred in 2 to 5 hours. The reactions were recorded in terms of the diameters of the areas of edema.

Results and Discussion. Electrophoretic analysis. Extraction and fractionation were carried out on 2 lots of *Trichinella* larvae. The electrophoretic patterns of Lot 1, shown in Fig. 1, 2 and 3, are also typical of the patterns of Lot 2. The difference in the total concentration of the primary extracts may be attributed to possible variations in the extraction and in centrifugation.

In the patterns of the primary extract of *Trichinella* larvae (Fig. 1) 3 homogeneous peaks are discernible. The area between peaks 1 and 2 is not a separate component and cannot be defined. More than 60% of the total primary extract is represented by the heterogeneous mixture designated as IV. The mobility calculated for this mass is an average value (Table I).

The patterns of the acid-soluble fraction are shown in Fig. 2. Three electrophoretically separable components were present, in addition to the heterogeneous mixture which had the highest mobility and an incompletely separated portion migrating between components 2 and 3. Physico-chemical changes produced by the acid treatment may be noted by comparing Fig. 1 and 2. The mobility and concentration of component 1 in the acid-soluble fraction are not significantly different from those of the corresponding component 1 in the primary extract. There were modifications in component 2 and the portion closely associated with it as shown by changes in concentration and in the mobility of the homogeneous peak. The most significant effect of the acid treatment is noted in the fast moving heterogeneous mass designated as IV. Computations of concentrations using

TABLE I.
Electrophoretic Analysis of Extracts of *Trichinella* Larvæ.

	Mobility, $\mu \times 10^5$ cm^2				Relative composition, % total areas				Concentration, g/100 ml				
Fraction	1	2	3	IV	1	2	3	IV	Total	1	2	3	IV
Primary extract													
Lot 1	2.1	4.2	5.8	7.8	6	13	15	62	4.00	0.24	0.52	0.61	2.48
" 2	1.8	4.7	5.9	8.0	12	5	13	65	2.76	0.32	0.14	0.36	1.80
Acid-soluble													
Lot 1	1.7	3.4	5.9	7.2	27	16	24	9	1.23	0.33	0.20	0.29	0.11
" 2	1.4	2.7	5.4	7.5	27	16	36	6	1.58	0.43	0.25	0.57	0.10
Heat-treated													
Lot 1	1.4	3.3	5.5	6.6	33	12	27	8	0.67	0.22	0.08	0.18	0.05

TABLE II.
Intradermal Reactions of Fractions of Larvæ of *Trichinella spiralis*.

Fraction	Size of reaction (mm)			Ratios of areas		Ratio of concentrations
	Smallest	Largest	Mean	Mean	Standard error	
			7 series of tests in rabbits.			
Primary extract	10 x 10	15 x 20	13.0 x 14.9	—	—	—
Acid-soluble	11 x 12	16 x 18	13.0 x 15.0	1.05	0.10	0.31
Heat-treated	7 x 10	14 x 15	11.0 x 12.6	0.74	0.08	0.17
			25 series of tests in guinea pigs.			
Primary extract	8 x 9	16 x 18	12.0 x 13.4	—	—	—
Acid-soluble	8 x 10	18 x 18	12.0 x 13.0	0.99	0.04	
Heat-treated	7 x 8	16 x 16	11.0 x 11.8	0.83	0.04	

TABLE III.
Relation of Concentrations of Components to Skin Reactions.

Fractions of Lot 1	Ratios of areas		Ratio of the concentrations of components		
	Rabbits	Guinea pigs	Component 1	Component 2	Component 3
Acid-soluble	1.05	0.99	1.4	0.4	0.5
Heat-treated	0.74	0.83	0.9	0.15	0.3

relative areas and dry weight determinations show that the material removed by acid treatment may be accounted for primarily by the decrease in portion IV.

The boundaries in the electrophoretic patterns of the heat-treated sample in Fig. 3 are very similar to those in patterns of the acid-soluble fraction. Three components separated and the 2 heterogeneous portions are present in the same positions as in Fig. 2. About 80% of the primary extract was removed by heating. Component 1 was apparently stable at the temperature and time interval used; there was some decrease in components 2 and 3 but the marked decrease in the heterogeneous mass IV was equivalent to approximately 75% of the total loss.

Intradermal reactions. Seven rabbits and 16 guinea pigs were injected with each of the 3 allergens. Prior to feeding of larvae, the rabbits did not respond to injections of primary extract. To confirm the specificity of the response, 14 normal guinea pigs were injected with each of the 3 allergens; there were no reactions. The saline control induced transitory responses in 2 of the 16 infected guinea pigs. The reactions were 3 × 3 mm, appeared later and persisted a shorter time than those to the fractions of larvae. Twenty-five series of tests were made on the infected guinea pigs with alternation in the location of injections.

The individual variations among the infected animals in response to an allergen were approximately the same when any one of the allergens was tested. In Table II are recorded the areas of smallest, the largest and the average reaction in the animals. The ratio of the edematous areas produced by the allergens and the ratio of dry weights were calculated by using the primary saline extract as the reference standard.

The acid treatment of the primary extract of the larvae produced no detectable alterations in properties which elicit dermal responses although 70% of the material had been removed. The electrophoretic patterns showed a significant decrease only in the heterogeneous mass designated as IV.

There was an indication of some decrease in the area of intradermal reactions produced from the injection of the antigen prepared by heating the primary extract. Most of fraction IV, as well as some of components 2 and 3, was removed by heating.

Computed on the basis of dry weight of soluble material present, the dilutions of the samples tested in sensitized rabbits were: Primary extract, 1:20,000, acid-soluble, 1:60,000; and heat-treated, 1:100,000. The decrease in the area of allergic reaction to the heat-treated fraction was relatively small in comparison to the dilution factors.

In Table III the concentration of each com-

ponent is compared with the skin activity of each fraction. The ratio of the concentration in grams per 100 ml (Table I) of each component was calculated by using the amount of each component in the primary extract as the reference standard for that component. If the ratios of concentration of the components are compared with the ratios of the areas of reactivity in skin tests (Table II) it will be noted that the concentration of component 1 parallels results of skin tests on the fractions. The ratios of the concentrations of components 2 and 3 and of skin activity are not proportional. These calculations indicate a stability of component 1 and suggest that the allergen is associated with this component.

Summary. A saline-soluble portion of larvae of *Trichinella spiralis* has been compared as to its electrophoretic characteristics and production of allergic response in sensitized animals with other fractions, prepared by acidification and by heating. Each of the 3 fractions separated into 3 electrophoretic components in addition to an incompletely separated portion associated with component 2 and a fast moving heterogeneous mixture. In the primary extract the heterogeneous mass represented more than 60% of the total concentration. Either acid or heat treatment removed practically all of this mixture. More material was precipitated by heating than by acidification. The 3 fractions were diluted on the basis of the original dry weight of larvae and intradermal reactions compared. There was no apparent difference in the area of edema produced by the primary extract and the acid-soluble fraction. The heat-treated allergen caused slightly less reaction than the other 2. There is an indication that the allergen is associated with component 1.

15409

Purified Rations and the Importance of Folic Acid in Mink Nutrition.*

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Domestication of mink has introduced many practical nutritional problems. The use of purified rations which has elucidated practical feeding problems in many other species of animals appears to offer a useful tool in the study of these problems in mink. Establishment of nutritional requirements of an animal so different from those previously studied would also be of considerable academic interest. In this paper we wish to present data on the use of purified rations and the requirement of folic acid for the mink.

Experimental. Previous to being placed on experiment the mink were housed in individual outdoor wire pens. They were fed a ration containing 72% horsemeat (including bone), 10% fresh liver, 12% cereal mixture, and 6% tomato puree. When the animals were placed on experiment the cages were moved into an unheated room and the nest boxes removed. The composition of the purified rations employed is given in Table I. The basal ration was similar to that used for dogs by Schaefer *et al.*[1] All rations were weighed into pound butter crocks, fitted with inverted conical shields in order to reduce spillage. Weekly weight and daily food consumption data were recorded. Blood samples were taken by severing the metatarsal vein according to the technic described by Kennedy.[2] White blood cell counts were determined by the

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1 Schaefer, A. E., McKibbin, J. M., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 365.

2 Kennedy, A. H., *Ontario Gov't. Exp. Fur Farm, Bul. No. 6*, 1933.